Protocol:

A Phase I/IIa, Randomized, Double-Blind, Placebo-Controlled, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Unilateral Intratympanic PIPE-505 in Subjects with Sensorineural Hearing Loss Associated with Speech-in-Noise Impairment

Amendment 3, Version 4.0 (18 September 2020)

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INVESTIGATOR SIGNATURE OF AGREEMENT PAGE

Protocol Title: A Phase I/IIa, Randomized, Double-Blind, Placebo-Controlled, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Unilateral Intratympanic PIPE-505 in Subjects with Sensorineural Hearing Loss Associated with Speech-in-Noise Impairment

Protocol Number: PTI-505-101

Protocol Version and Date: Version 4.0 (18 September 2020)

I have read this protocol in its entirety.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the Sponsor. It is, however, permissible to provide information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Conference on Harmonisation Guidelines on Good Clinical Practice and with the applicable regulatory requirements. I will inform all who assist me in the conduct of this study of their responsibilities and obligations. Furthermore, I understand that the Sponsor and the Institutional Review Board/Ethics Committee (IRB/IEC) must approve the protocol and any changes to the protocol in writing before implementation, unless a deviation is required to eliminate an immediate safety hazard to a subject. In such cases, I will notify the Sponsor and the IRB/IEC as soon as possible.

I understand that failure to comply with the requirements of the protocol may lead to my participation as an Investigator for this study to be terminated.

Principal Investigator Name: _____

Institution: _____

Signature: _____ **Date:** _____

[redacted]

EMERGENCY CONTACT INFORMATION

In the event of a Serious Adverse Event (SAE), the Investigator must fax or e-mail the Serious Adverse Event Form within 1 business day to the following:

Table 1: SAE and Pregnancy Reporting Contacts

[redacted]

For protocol or safety related issues other than SAE reporting **during normal business hours (8 am – 5 pm ET)**, contact the Medical Monitor:

[redacted]

For protocol - or safety-related issues **outside of normal business hours (5pm – 8am ET)**, the Investigator must contact the following:

[redacted]

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ABBREVIATIONS

ABR	Auditory brainstem response
AE	Adverse event
ANF	Auditory nerve fiber
AP	Action potential
AUC	Area under the curve
BMI	Body mass index
BP	Blood pressure
CFR	Code of Federal Regulations
CL/F	Apparent clearance
C_{max}	Maximum plasma concentration
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organization
CS	Cochlear synaptopathy
daPa	DekaPascals
dB	Decibel
DCC	Deleted in colorectal cancer
DMC	Data Monitoring Committee
DPOAE	Distortion product otoacoustic emission
ECG	Electrocardiogram
ECochG	Electrocochleography
EMA	European Medicines Agency
FAS	Full analysis set
FDA	Food and Drug Administration
FOCP	Females of child-bearing potential
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HCG	Human chorionic gonadotropin
HED	Human equivalent dose
HHL	Hidden-hearing-loss
HIPAA	Health Insurance Portability and Accountability Act
Hz	Hertz
IB	Investigator's Brochure

[redacted]

ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IEEE	Institute of Electrical and Electronics Engineers
IHC	Inner hair cells
IP	Investigational product
IRB	Institutional Review Board
IUD	Intrauterine device
IxRS	Interactive Voice/Web Response System
MCL	Most Comfortable Level
MedDRA	Medical Dictionary for Regulatory Activities
MEMR	Middle Ear Muscle Reflex
MHRA	Medicines and Healthcare Products Regulatory Agency (UK)
mL	Milliliter
Mmho	Millimhos
MMRM	Mixed model with repeated measurements
MoCA	Montreal Cognitive Assessment
MRSD	Maximum Recommended Safe Dose
nHL	Normalized hearing level
NHP	Nonhuman primates
NOAEL	No Observed Adverse Effect Level
OHC	Outer hair cells
PIPE-505	Investigational Product
PK	Pharmacokinetic
PPS	Per-protocol set
QuickSIN	Quick Sentences-in-Noise
RBC	Red blood cell
ROA	Route of administration
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SGN	Spiral ganglion neurons
SNHL	Sensorineural hearing loss
SNR	Signal-to-noise ratio
SOC	System organ class
SOE	Schedule of events

SOP	Standard operating procedure
SP	Summating potential
SR	Spontaneous rate
SRT	Speech Recognition Threshold
SSQ	Speech, Spatial and Qualities
$t_{½}$	Apparent terminal-phase disposition half-life
TEAE	Treatment-emergent adverse
t_{max}	Time to maximum plasma concentration
TM	Tympanic membrane
TTS	Temporary threshold shifts
WBC	White blood cell
WIN	Words in Noise
WHO	World Health Organization
WRS	Word Recognition Score
V_z/F	Apparent volume of distribution
λ_z	Terminal elimination rate constant

[redacted]

STUDY SYNOPSIS

Protocol number: 505-101	**Drug:** PIPE-505

Title of the study:

A Phase I/IIa, Randomized, Double-Blind, Placebo-Controlled, Safety, Pharmacokinetic, and Preliminary Efficacy Study of Unilateral Intratympanic PIPE-505 in Subjects with Sensorineural Hearing Loss Associated with Speech-in-Noise Impairment

Number of subjects (total and for each treatment arm):

Approximately 24 subjects will be enrolled (18 Active: 6 Placebo)

Investigators/Sites: Approximately 7 sites in the US

Clinical phase: Phase I/IIa

Objectives:

Primary: To assess the safety and tolerability profile of PIPE-505 when administered as a single unilateral intratympanic injection

Secondary: To assess the single dose plasma PK profile of PIPE-505 following a single unilateral intratympanic injection

Exploratory:

- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on auditory skills requiring signal-to-noise processing and encoding.
- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on hearing thresholds and outer hair cell function.
- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on auditory electrophysiological measures.

Investigational product, dose, and mode of administration:

Separate vials of PIPE-505 in crystalline powder form and a Diluent consisting of a sterile thermoreversible gel (Poloxamer 407) will be provided to the sites for local formulation of the Suspension according to a dose concentration of 0.75%. The Diluent alone will serve as the Placebo.

PIPE-505 or Placebo will be administered in a volume of 200 microliters via a unilateral intratympanic injection targeting the round window niche. The round window membrane will permit sustained release diffusion into the cochlea with negligible systemic exposure. At the discretion of the investigator, local anesthesia may be administered topically to the tympanic membrane (TM) prior to study drug administration.

Methodology:

The Phase I/IIa investigation will be conducted as a randomized, double-blind, placebo-controlled, single dose study with approximately 24 subjects (18 Active: 6 Placebo). Subjects who provide written informed consent and meet eligibility criteria will be randomized to receive a single intratympanic dose of PIPE-505 Suspension or Placebo (Poloxamer 407). A sentinel dosing strategy will be employed such that the first 2 subjects (1:1, PIPE-505:Placebo) will be dosed and complete the Day 1 assessment prior to dosing the remaining subjects. The PIPE-505 or Placebo injection will be administered into the ear with the worse audiometry results (i.e., the Index Ear, additional criteria apply, refer to Section 6.3.1).

The primary, secondary and exploratory endpoints will be measured over the course of 3 months following dose administration.

[redacted]

The safety assessments include vital signs, electrocardiograms (ECG), clinical laboratory test results (routine hematology, chemistry, and urinalysis), physical and otoscopic examinations, AEs/SAEs, audiometry, and tympanometry.

The preliminary efficacy assessments include audiometry, distortion product otoacoustic emission, auditory brainstem responses, electrocochleography (ECochG), middle ear muscle reflex, Speech Spatial Qualities of Hearing Scale, Word Recognition Score (WRS), Speech Recognition Threshold (SRT), Quick Sentences-in-Noise (QuickSIN), and Words in Noise (WIN).

Eligibility Criteria:

Subjects must meet each of the following inclusion criteria to be eligible for randomization:

1. Ability to personally provide written, signed, and dated informed consent to participate in the study.
2. Subject's primary language is English.
3. Male or female between 18 and 75 years of age, inclusive, at randomization.
4. Diagnosis of bilateral sensorineural hearing loss (SNHL) with air conduction threshold elevation by pure tone audiometry that is no greater than 55 dB at 250, 500, and 1000 Hz, no greater than 65 dB at 2000 Hz, and no greater than 85 dB at 4000 and 8000 Hz at Screening in either ear.
5. Threshold on the QuickSIN-EP > 4 dB SNR Loss at Screening in at least one ear.
6. Normal tympanogram in the ear intended for injection (i.e., the Index Ear), as guided by a range of -140 to +40 dekaPascals [daPa] and 0.3-2.9 millimhos [mmho]) at Screening, and as determined by the Investigator.
7. Male or female subjects with reproductive potential agree to comply with approved double barrier contraceptive method (e.g., condom plus intrauterine device [IUD], condom plus hormonal contraception, or double barrier method, i.e., condoms and diaphragms with spermicidal gel or foam) during and for 3 months after study drug administration.

   Subjects are considered of non-reproductive potential if:

   a. Post-menopausal female with ≥12 consecutive months of spontaneous amenorrhea and age ≥51 years with follicle-stimulating hormone (FSH) >30 mIU/mL at Screening
   b. Surgically sterile female and at least 6 weeks post-sterilization (i.e., bilateral oophorectomy or hysterectomy)
   c. Sterilized male at least 1-year post vasectomy and confirmed that they have obtained documentation of the absence of sperm in the ejaculate
8. The subject is in general good medical health with no clinically significant or relevant abnormalities, including medical history, physical exam, vital signs, ECG, and laboratory evaluations (hematology, chemistry, and urinalysis) as assessed by the Investigator.
9. An understanding, ability, and willingness to fully comply with study procedures and restrictions.

Subjects are excluded from the study if any of the following exclusion criteria are met:

1. Montreal Cognitive Assessment (MoCA) score less than 26 at Screening.
2. History of chronic otitis externa or media, other chronic middle ear disorders, or perilymph fistula.
3. Presence of a genetic, syndromal or developmental auditory disorder.
4. Presence of an autoimmune or serious neurological disorder that could contribute to auditory loss, as determined by the Investigator.
5. History of herpes zoster oticus, or other infectious etiology of hearing loss as determined by the Investigator.
6. History of barotrauma as determined by the Investigator.

[redacted]

7. Evidence of current conductive hearing loss, mixed hearing loss or otosclerosis as determined by the Investigator including significant air-bone gaps (i.e., >10 dB on audiometric testing, with the exception of 4000 Hz).
8. History of auditory loss related to exposure to known ototoxic drugs, such as high-dose aminoglycoside antibiotics (e.g., streptomycin, gentamicin, tobramycin, amikacin, neomycin, or minocycline), as determined by the Investigator.
9. Any prior exposure to platinum-based medications.
10. Otological disorders that would preclude safe tympanic injection (e.g., history or presence of cholesteatoma, TM perforation, otitis media, or middle ear anatomic anomaly) as determined by the Investigator.
11. Presence of a cochlear implant.
12. History of Meniere's disease or endolymphatic hydrops.
13. History of bothersome tinnitus, as determined by the Investigator.
14. Past otologic surgical procedures that would preclude safe intratympanic injection as determined by the Investigator.
15. Intratympanic injection within 6 months of randomization.
16. Use of an investigational product or intervention other than a non-interventional registry study (including vaccine studies) within the greater of 30 days or 5 half-lives (if known) prior to Screening or expected during the study.
17. Asymmetrical audiometric threshold results >15 dB between ears at any octave frequency between 250 and 8000 Hz at Screening AND the pattern of asymmetry is inconsistent with SNHL per Investigator judgment.
18. History of malignancy under current active treatment or considered at substantial risk for progression or recurrence during the study interval, as determined by the Investigator. Note, central nervous system neoplasms or head and neck cancer are excluded from eligibility regardless of treatment status.
19. The subject has a QTcF by ECG > 450 milliseconds for males or >470 milliseconds for females.
20. The subject has a history of dysrhythmia (such as atrial fibrillation or ventricular tachycardia) that is considered unstable by the Investigator.
21. The subject has experienced a significant systemic illness, as judged by the Investigator, within 30 days prior to Screening.
22. The subject has a current or relevant history of physical or psychiatric illness, or any medical disorder that may require treatment or make the subject unlikely to fully complete the study, or any condition that presents undue risk from the investigational product or procedure, as determined by the Investigator.
23. Known or suspected intolerance or hypersensitivity to the investigational product or closely-related compounds.
24. The subject is pregnant or breastfeeding or plans to become pregnant during the study.
25. History of alcohol or other substance abuse within 6 months of Screening as determined by the Investigator.
26. Subject is Hepatitis B positive (subjects who are surface antibody positive secondary to immunization are eligible).
27. History of Hepatitis C.
28. Subject is Human Immunodeficiency Virus (HIV) seropositive.

[redacted]

Duration of subject involvement in the study:

- Screening period: up to 8 weeks
- Treatment period: single intratympanic injection on Day 0
- Follow-up period: 12 weeks
- Total duration of subject participation: up to 20 weeks

Statistical Considerations:

Study Endpoints

- The primary endpoint is the number and severity of adverse events (AEs) up to 3 months following a single unilateral intratympanic injection of PIPE-505.
- The secondary endpoint of the study is the single dose plasma PK parameters of PIPE-505, including AUC_t, AUC_{24}, AUC_∞, C_{max}, t_{max}, $t_{½}$, CL/F, V_z/F, λ_z.
- Exploratory Endpoints will assess efficacy as follows:
  - Mean change from baseline to 3 months in WRS (performed in quiet), QuickSIN (monaural and binaural), Speech Spatial Qualities (SSQ), SRT, and WIN tests
  - Mean change from baseline to 3 months in pure tone audiometry and distortion product otoacoustic emission (DPOAE)
  - Mean change from baseline to 3 months in Middle Ear Muscle Reflex (MEMR), Auditory Brainstem Response (ABR), and ECochG

Statistical Analyses & Sample Size justification

The sample size of this study is not based on statistical considerations. A sample size of 24 subjects (18:6 PIPE-505:Placebo) is considered sufficient to characterize the initial safety, tolerability, single dose pharmacokinetic (PK) profiles, and exploratory preliminary efficacy endpoints as measured by clinical behavioral and electrophysiological responses.

Safety and preliminary efficacy results from this study will be assessed using summary statistics (e.g., n, mean, median, minimum, maximum, for continuous endpoints; n and % for binary and categorical endpoints). AEs will be presented in listings by system organ class (SOC) and summarized by treatment, relatedness, and severity. Results of vital signs, ECG, and laboratory results will be listed and change from baseline values will be summarized using shift tables.

PK parameters will be summarized using descriptive statistics (n, arithmetic means, geometric means, standard deviation, % coefficient of variation, median, minimum, and maximum). Figures will be created to display mean and individual subject PIPE-505 concentration time curves in plasma on both a linear and logarithmic scale.

[redacted]

SCHEDULE OF EVENTS

Table 2: Schedule of Events

Procedure	Visit	Screening	1	2	3	4	5	6	7
	Day	-56 to -1	0	1	7	14	30	60	90[a]
	Window	-	-	-	+/- 2	+/-2	+/-3	+/-7	+/- 7
Informed Consent		X							
Inclusion/Exclusion Criteria		X							
Demographics		X							
Height		X							
Medical and Medication History		X	X						
Physical Examination		X							X
Otological Examination[b]		X	X	X	X	X	X	X	X
Weight		X							X
Vital Signs[c]		X	X[d]	X	X	X	X		X
ECG[e]		X	X[f]	X	X				X
FSH[g]		X							
Pregnancy Test[h]		X[h]	X[h]						X[h]
Hep B, Hep C, HIV		X							
Urinalysis		X							
Coagulation Panel		X							
Hematology		X	X[i]	X	X	X	X	X	X
Serum Chemistry		X	X[i]	X	X	X	X	X	X
PK Blood Collection			X[j]	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X
AEs		X	X	X	X	X	X	X	X
Study Drug (PIPE-505) or Placebo Admin			X[k]						
TRQ		X							X
MoCA		X							
Audiometry (with ultra-high frequency[l])		X					X	X	X
DPOAE		X					X		X
ABR/ECochG		X					X		X
Tympanometry		X					X		X
MEMR		X					X		X
SSQ		X							X
WRS		X					X	X	X
SRT		X					X	X	X
QuickSIN-EP (monaural via earphones)		X					X	X	X
WIN (monaural via earphones)		X							X
QuickSIN (binaural via sound field)[m]		X					X		X

Footnotes:

[a] Subjects who receive study drug and withdraw or are withdrawn from the study early will be requested to complete early termination procedures outlined under Day 90.

[b] Otological examination will assess the following under direct magnification: external ear, external auditory canal, TM and TM mobility. The otological examinations will be conducted by the unblinded otologist who performs the intratympanic injection and also conducts all post-injection otological examinations through at least Day 30 and preferably throughout the study.

[c] Vital signs (i.e., blood pressure, pulse rate, respiratory rate, and temperature) will be recorded after subject is at rest in a seated or supine position for at least 5 minutes.

[d] On Day 0, vital signs will be conducted pre-dose, 30 (±5) minutes post-dose, and 4 hours (±15 minutes) post-dose.

[e] ECG will be recorded after subject is at rest in a supine position for at least 5 minutes.

[f] On Day 0, ECG will only be conducted at 4 hours (±15 minutes) post-dose.

[g] FSH level will be obtained on post-menopausal women only (defined as continuous amenorrhea of at least 12 months and not surgically sterile).

[redacted]

[h] Only female subjects of childbearing potential will require a negative serum pregnancy test at Screening and negative urine pregnancy test prior to randomization at Day 0. Female subjects of childbearing potential will also require a urine pregnancy test at Completion or Early Termination.
[i] On Day 0, hematology and chemistry lab samples will be collected prior to dosing but do not have to be analyzed prior to dose.
[j] On Day 0, PK samples will be collected pre-dose and 4 hours (±15 minutes) post-dose.
[k] The intratympanic injection will be performed by an unblinded otologist who will also conduct all post-injection otological examinations through at least Day 30 and preferably throughout the study.
[l] Ultra-high frequency audiogram testing [10,000-16,000 Hz] will be performed if available based on site audiometer specifications.
[m] Sound field binaural testing using a single 0^0 azimuth loudspeaker.

[redacted]

1. INTRODUCTION

1.1 Background

Recent studies in animal models have demonstrated that acoustic damage related to noise and/or aging can result in degeneration of the synaptic contacts between the spiral ganglion neurons (SGN) and the inner hair cells (IHC) of the cochlea (Kujawa and Liberman, 2015). The synaptic degeneration is now recognized to precede IHC and/or outer hair cells (OHC) loss and has been demonstrated in animal models exposed to only minor auditory insults, also referred to as temporary threshold shifts (TTS). Research in animal models has also indicated that not only is the synapse between the SGN and IHC, also referred to as the cochlear synapse, primarily responsible for encoding complex auditory signals, but it may be specifically vulnerable to degeneration independent of inner and outer hair cells. Results from recent animal studies strongly suggest that degeneration of human SGN synaptic function (i.e., cochlear synaptopathy [CS]) may significantly contribute to the impairment in speech perception in the setting of background noise, while sensitivity to sound processing in quiet settings is not disrupted (Plack et al., 2016). It has been suggested that the disproportionate innervation of each IHC by multiple SGNs facilitates selective differentiation of weak signals of importance to the listener from the background of competing stimuli (Kujawa and Liberman, 2019). The inability to hear and understand speech in challenging noise backgrounds in everyday settings is commonly reported by patients with sensorineural hearing loss (SNHL), and to date there is no effective treatment for restoring this specific auditory function (Kobel et al., 2017).

Emerging data from animal studies focused on the cochlear synapse support the hypothesis that deafferentation of IHCs is a common pathology across the spectrum of human SNHL etiologies, regardless of the degree of overt hearing loss as measured by audiometry (Liberman and Kujawa, 2017). Animal investigations have revealed that noise exposure causing TTS, previously thought to be innocuous, can produce a loss of as many as 50% of the synapses and cochlear nerve terminals innervating the IHC, resulting in pronounced CS (Kujawa and Liberman, 2009; Bharadwaj et al., 2015; Lin et al., 2011; Lobarinas et al., 2016; Hickox et al., 2017). Evidence of noise-induced CS has also been observed in the guinea pig (Lin et al., 2011; Furman et al., 2013), chinchilla (Hickman et al., 2018), and rat (Singer et al., 2013), as well as normal aging (Sergeyenko et al., 2013; Altschuler et al., 2015; Möhrle et al., 2016) and administration of ototoxins (Bourien et al., 2014; Li et al., 2016).

Collective animal data has further indicated that the Type I synapse, in particular the low spontaneous rate (SR) neurons, may be a particularly vulnerable link in the auditory nerve circuit and related hearing function (Hickox et al., 2017). Normal response properties of low SR neurons, in quiet and in noise, have led to speculation regarding functional consequences of their targeted loss in humans and the contribution to the classic impairment of speech-in-noise difficulty seen in SNHL (Kujawa and Liberman, 2015; Plack et al., 2014). The auditory properties and function of the low SR neurons may be particularly relevant to declines in performance with age that include decreased speech understanding in noise and reduced ability to utilize spatial stimulus timing and amplitude modulation cues (Schmiedt et al., 1996). CS also may be a key elicitor of tinnitus and hyperacusis, two of the most troubling sensory symptoms associated with SNHL.

Finally, the emerging electrophysiological and histological animal data have also paralleled recent human studies where both electrophysiological measures and post-mortem analyses are consistent with human CS, including temporal and spatial auditory processing (Mehraei et al., 2016; Bharadwaj et al., 2014; Wu et al., 2018). Electrophysiological testing has consistently demonstrated reduction in Wave I of the auditory brainstem response (ABR) in animal models of both noise-induced and age-related cochlear deterioration. Wave I originates with the synaptic component of the auditory nerve circuit and the ABR ultimately assesses the auditory circuit extending to the brain stem and temporal cortex. Similar reductions in Wave I amplitudes has been shown in select populations with known histories of auditory insults (Bramhall et al., 2017). Post-mortem studies have examined human temporal bone specimens in patients ranging from 55-89 years of age with demonstrated loss of synaptic connections with the IHC ranging from 15 synapses per IHC in younger patients to 2.5 synapses per IHC in older patients - without major loss of IHC or OHCs in the same temporal bones (Wu et al., 2018). More importantly, these post-mortem studies have also demonstrated survival of viable SGN, indicating that the primary sensory neuron remains intact and is potentially responsive to synaptic restoration.

Synaptic degeneration is now proposed as a major contributor to the classic speech-in-noise impairment associated with SNHL (Kujawa and Liberman, 2015; Plack et al., 2014; Bharadwaj et al., 2015; Le Prell and Brungart, 2016; Le Prell, 2019). Based on experimental work in noise-induced and ageing rodent models of hearing loss, the leading functional outcome of synaptic degeneration in humans is hypothesized to produce difficulty with listening in noisy backgrounds (Bramhall et al., 2019). CS may be one of the earliest manifestations of future SNHL wherein both hair cell loss and synaptic degeneration co-exist, the latter of which may selectively impact speech perception in complex listening conditions. Results in animal studies have further indicated that the synaptic connection between the auditory nerve fibers (ANF) and IHCs are the most vulnerable element within the cochlea, and degenerate before the loss of hair cells. However, the long-term survival of the SGN and the central axon in humans underlies the effectiveness of cochlear implants (Kujawa and Liberman, 2019). The age-related synaptic degeneration demonstrated in post-mortem temporal bone studies parallel the findings in age-related CS in multiple animal models. The predictability of the animal models is further supported by the age-related changes in ABR Wave I morphology seen in both rodent and human subjects (Le Prell, 2019). Finally, correlation with performance on speech-in-noise assessments (e.g., the Quick Sentences in Noise [QuickSIN], the Words-in-Noise test [WIN]) and ABR Wave I amplitude have also been recognized in humans, further indicating the role of the cochlear synapse in complex speech perception and speech-in-noise impairment (Le Prell, 2019). Thus, repair of the cochlear synapse may lead to improved speech-in-noise processing and address one of the main disabilities associated with SNHL.

1.2 The Condition Background and Current Treatment

SNHL is the major cause of hearing impairment in adults, comprising both noise-induced and age-related auditory disorders. It is estimated that about two-thirds of people over the age of 70 have a degree of age-related hearing loss which can lead to social isolation and decreased quality of life (Heeringa and Köppl, 2019). The worldwide prevalence of disabling hearing loss is thought to exceed 5% according to the World Health Organization (WHO), and the majority of cases (possibly up to 90%) are classified as sensorineural (Nyberg et al., 2019). The underlying cochlear

histopathology has historically been characterized as loss of the primary sensory cells (inner and outer hair cells), but as noted above the cochlear synapse may be even more vulnerable to noise exposure and aging. The synaptic connection between the auditory nerve and the IHC may play a primary role in the normal processing of complex auditory stimuli, and functional auditory deficits secondary to degeneration of the synapse may be comparable to the impairment related to OHC loss.

Clinical manifestations of SNHL are typically characterized by elevation in hearing thresholds as measured by the pure tone audiometry, but audiometry alone does not assess the full spectrum of hearing perception difficulties reported by patients. While amplification may augment sound detection when hearing loss is severe, such as provided by hearing aids, processing more challenging auditory tasks such as understanding speech in the setting of background noise are not addressed by amplification alone. In that regard, a leading complaint among patients with SNHL is difficulty hearing conversations in noisy environments, since audiometric thresholds do not assess the full extent of hearing dysfunction involving speech perception-in-noise is not measured by audiometry, this auditory disability has also been referred to as hidden-hearing-loss (HHL).

In summary, degeneration of the cochlear synapse between the auditory nerve and IHC, i.e., CS, may reduce signal-to-noise processing in challenging listening environments. There is no current drug treatment for speech-in-noise impairment, and existing technologies do not ameliorate this specific domain of hearing function. However, treatment designed to restore synaptic function may lead to improved temporal, spatial, and signal-to-noise processing, and consequently address one of the major auditory disabilities in SNHL.

1.3 Product Background

PIPE-505 is a novel, intratympanically bioavailable inhibitor of gamma-secretase, a membrane bound enzyme regulating key pathways involved in inner ear development, including sensory hair cell development via Notch signaling and spiral ganglion outgrowth via netrin-deleted in colorectal cancer (DCC) signaling (reviewed in Haapasalo and Kovacs, 2011). The current pharmacological data support a key role for Notch signaling activity in OHC differentiation in the cochlea (Zheng et al., 2000; Zine et al., 2001; Yamamoto et al., 2006; Deyts et al., 2016; Brugead et al., 2014) and netrin-DCC signaling activity in neurite guidance and outgrowth at the SGN (Bai et al., 2011; Kim et al., 2016).

PIPE-505 regulates both Notch activity as well as DCC activity by binding to the gamma-secretase protease complex. PIPE-505 inhibits transmembrane cleavage and subsequent release of the Notch intracellular domain. As a result, genes activated by the Notch intracellular domain, e.g., Hes1, Hey1, Hey2, and Hes5 are downregulated. One consequence of Hes5 downregulation is the de-repression of the transcription factor Atoh1, which stimulates ear hair cell differentiation (Lin V et al., 2011). In addition, inhibition of gamma-secretase by PIPE-505 prevents cleavage of DCC on SGN, resulting in persistent expression of the receptor and consequent increases in axonal length toward IHCs where synaptic connections reform.

Based on the biological properties as a gamma-secretase inhibitor, PIPE-505 is currently under development for the treatment of SNHL associated with speech-in-noise impairment (potentially secondary to CS). Nonclinical *in vitro* studies and *in vivo* experiments with relevant animal models

(summarized in Section 1.3.1 below) have demonstrated the two distinct mechanisms-of-action as outlined above.

The Investigator's Brochure (IB) summarizes the most current information regarding the safety and efficacy of PIPE-505.

1.3.1 Preclinical Information

PIPE-505 has been evaluated in a series of nonclinical *in vitro* and *in vivo* studies in animal models with relevant auditory insults. As stated above, the preclinical studies have demonstrated two distinct mechanisms of action that may restore hearing function in SNHL. Specifically, 1) regeneration of synapses between Type I SGN and IHC via the netrin-DCC pathway, and 2) outer hair cell regeneration via the Notch pathway. The outcome of select studies are summarized below.

Within the auditory sensory nerve pathway and cochlea, the gamma-secretase complex cleaves and subsequently inactivates, membrane substrates which are involved in neurite outgrowth and pathfinding. DCC is a known substrate of gamma-secretase and the netrin-DCC complex has been implicated in axonal guidance (Lee and Warchol, 2008; Kim et al., 2016). Within the cochlea, DCC is expressed by SGN and netrin is expressed by IHCs (Shrestha et al., 2018). Inhibition of gamma-secretase prevents the cleavage of DCC, resulting in persistent expression of select receptors and consequent increased axonal length.

- *In vitro* studies using mouse disassociated cochlea culture demonstrated that inhibition of gamma-secretase dose dependently induces neurite outgrowth of Type I SGNs. Function blocking antibodies against the axon guidance pathway (i.e., DCC and netrin) inhibit PIPE-505 induced neurite outgrowth, indicating that neurite stimulation is generated by PIPE-505 via the netrin-DCC pathway and aids synapse regeneration.
- *In vivo* studies with TTS in a mouse model have produced synaptic degeneration which is reversed by treatment with PIPE-505 as demonstrated by restoration of synaptic histology and improvement in Wave I of the ABR.
- *In vivo* studies with a guinea pig ototoxicity model have also shown synapse recovery by histology that persists up to 3 months post-injection.

Gamma-secretase inhibition also leads to inhibition of Notch signaling, resulting in the de-repression of the Atoh1 enhancer element and subsequent induction of Atoh1 (a key regulator of outer hair cell differentiation) in supporting progenitor cells.

- *Ex vivo* studies with rodent cochlear explants have shown increased OHCs in both naïve models, as well as a model of ototoxicity secondary to gentamicin exposure.
- *In vivo* studies in a murine model of noise-induced hearing loss demonstrated improved ABR thresholds across 3 frequencies (16, 24, and 32 kHz) and mice with 10 dB improvement or more showed a strong trend of an average of 20% or more OHCs after a single intratympanic injection of PIPE-505.

[redacted]

Potential local and systemic toxicity of PIPE-505 (administered both orally and by intratympanic injection) has been tested in Good Laboratory Practice (GLP)-compliant repeat-dose studies in rats, guinea pigs, and monkeys. PIPE-505 was well-tolerated across all of the animal studies, and no systemic or local safety events were observed. In summary, the No Observed Adverse Effect Level (NOAEL) was identified at the local intratympanic dose level of 5.0% in nonclinical studies performed with guinea pigs.

This study will be the first-in-human investigation of PIPE-505, and therefore the pharmacokinetics (PK) and metabolism have not yet been measured in human subjects. Based on nonclinical studies in several different animal species, single unilateral intratympanic administration should result in measurable drug presence within the cochlea perilymph for at least one month after injection. Systemic exposure may primarily result via the Eustachian tube and subsequent absorption through the gastrointestinal route. Nonclinical studies have shown that the amount of systemic exposure following an intratympanic administration is very limited and restricted to within 7 days of drug administration (refer to the IB for a summary of the nonclinical PK studies). Human PK will be assessed based on the collection plan as outlined in the Schedule of Events (SOE) for all subjects dosed with PIPE-505.

As noted above, no human data with PIPE-505 has been collected, but nonclinical studies indicate that restoration of the cochlear synapse and generation of new OHCs could improve auditory function in subjects with SNHL with speech-in-noise impairment (secondary to suspected CS). The physiology and function of the human cochlear synapse indicates that the therapeutic effect of PIPE-505, and thereby preliminary efficacy (refer to exploratory objectives and endpoints), could include improved signal-to-noise processing. This treatment effect will be detected by improved performance from baseline on the QuickSIN and/or the WIN, specific speech-in-noise measures that test the ability of the subject to hear speech or words in the setting of competing background noise. Other hearing measures that could reflect evidence of preliminary efficacy include the Word Recognition Score (WRS), Speech Recognition Threshold (SRT), and Speech Special Qualities (SSQ). Improved cochlear synaptic function may also be detected by electrophysiological changes in the ABR, electrocochleography (ECochG), and/or the Middle Ear Muscle Reflex (MEMR). Finally, the treatment effect, potentially related more to OHC regeneration, could also be reflected in changes from baseline in pure tone audiometry and distortion product otoacoustic emission (DPOAE).

Given the local, and unilateral, administration of PIPE-505 directly into the tympanic cavity as a single dose, a favorable systemic safety profile is anticipated. Expected adverse events (AEs) are likely to be related to the effects of intratympanic injection and have been observed in other studies involving the same route-of-administration (Meyer, 2013; Staecker et al., 2017). The more common otologic/audiologic responses associated with tympanic injection may include transient vertigo, ear pain, dizziness, nausea, ear fullness or transient reduction in hearing. The expected AEs related to this study and PIPE-505 are fully summarized in the IB.

The dose of PIPE-505 in this study is based on the NOAEL established in nonclinical studies (as summarized above) and the relevant Human Equivalent Dose (HED) conversion derived from the allometric difference in perilymph volume from the guinea pig to the human. A ten-fold safety factor (HED/10) is then applied to the HED to calculate the Maximum Recommended Safe Dose (MRSD). Based on the nonclinical studies (summarized in the IB), the MRSD calculated for this

study is a 0.75% PIPE-505 Suspension in 200 microliters (i.e., 1.5 mg of active ingredient). Additional details regarding the nonclinical pharmacology and toxicity studies are summarized in the current version of the IB.

1.4 Rationale for the Study

Nonclinical efficacy studies have demonstrated the potential for intratympanic administration of PIPE-505 to restore cochlear synaptic function and to regenerate OHCs in animal models with induced cochlear dysfunction. Specifically, the cochlear synapse is a key element in signal-to-noise processing known to be diminished in both noise-induced and age-related SNHL populations. Difficulty with speech-in-noise is one of the most common complaints of patients with hearing loss, and there are no current approved treatments for this deficit in auditory function. Regeneration of cochlear synaptic function may improve signal-to-noise auditory processing and thereby speech-in-noise comprehension, addressing one of the major perceptual disabilities associated with SNHL.

The primary objective of this first-in-human study is to assess the safety and tolerability of PIPE-505 when administered as single unilateral intratympanic injection. Preliminary efficacy will be explored by changes from baseline in auditory performance as assessed by speech-in-noise tests and electrophysiological measures linked to cochlear synaptic function. Collectively these data, in addition to systemic PK exposure, will inform the design of future clinical studies with PIPE-505 in subjects with SNHL associated with speech-in-noise hearing impairment.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objectives

To assess the safety and tolerability profile of PIPE-505 when administered as a single unilateral intratympanic injection.

2.1.2 Secondary Objectives

To assess the single dose plasma PK profile of PIPE-505 following a single unilateral intratympanic injection.

2.1.3 Exploratory Objectives

- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on auditory skills requiring signal-to-noise processing and encoding.
- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on hearing thresholds and outer hair cell function.
- To explore the effect of a single unilateral intratympanic injection of PIPE-505 on auditory electrophysiological measures.

2.2 Endpoints

2.2.1 Primary Endpoint

The primary endpoint of the study is the number and severity of AEs up to 3 months following a single unilateral intratympanic injection of PIPE-505.

2.2.2 Secondary Endpoints

Single dose plasma PK parameters of PIPE-505, including AUC_t, AUC_{24}, AUC_∞, C_{max}, t_{max}, $t_{½}$, CL/F, V_z/F, λ_z).

2.2.3 Exploratory Endpoints

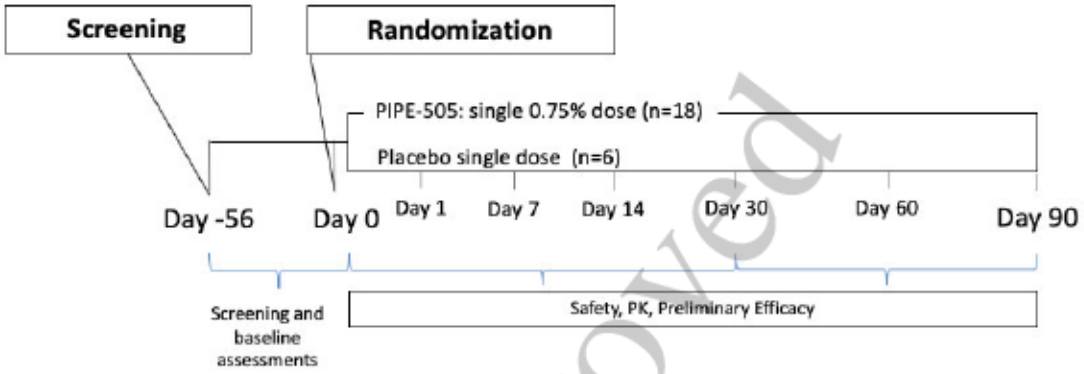
- Mean change from baseline to 3 months in WRS (performed in quiet), QuickSIN, SSQ, SRT, and WIN tests
- Mean change from baseline to 3 months in pure tone audiometry and DPOAE
- Mean change from baseline to 3 months in MEMR, ABR and ECochG

3. STUDY DESIGN AND DESCRIPTION

3.1 Study Design

This is a Phase I/IIa randomized, double-blind, placebo-controlled, single dose study in subjects with SNHL associated with speech-in-noise impairment. Figure 1 provides a schematic overview of the study design.

Figure 1: 505-101 Study Schematic



Approximately 24 adults will be enrolled into a single dose cohort comprised of 18 Active and 6 Placebo subjects according to a 3:1 randomization. All study procedures will be performed in an outpatient setting. Subjects who provide written informed consent and meet eligibility criteria will be randomized to receive a single intratympanic dose of PIPE-505 or Placebo (18:6 PIPE-505:Placebo). The Placebo injection will contain the thermoreversible gel alone (Poloxamer 407).

Following confirmation of eligibility and completion of baseline assessments, subjects will be randomly assigned to receive either PIPE-505 or Placebo. The PIPE-505 or Placebo injection will be administered into the ear with the worse audiometry results (i.e., the Index Ear, additional criteria apply, refer to Section 6.3.1) as a single 200 microliter injection. The injection, targeting delivery near the round window niche, will be performed by an otologist or neuro-otologist under otoscopic visualization of the tympanic membrane (TM), and will be conducted as an out-patient procedure. Subjects will remain in the clinic until completion of the assessments at 30 minutes post-injection, after which the subjects may leave the clinic but must return to undergo the 4-hour post-injection assessments as per the SOE. Thereafter, subjects will return for visits and procedures according to the SOE described in Table 2.

The primary, secondary and exploratory preliminary efficacy endpoints will be measured for up to 3 months following dose administration.

Local administration of PIPE-505 into the tympanic cavity permits access to the cochlea via diffusion through the round and oval window, providing a route of delivery that concentrates the active product to the cochlea and perilymph, the target organ and tissue of interest (consistent with

the route of administration [ROA] investigated in nonclinical studies involving several species, including nonhuman primates [NHPs]). The local administration also minimizes systemic exposure, and the formulation in a thermosensitive gel facilitates longer exposure within the tympanic cavity, proximity to the round and oval window, and ultimately longer times to allow optimal diffusion into the cochlea.

3.2 Sentinel Dosing Plan

A sentinel dosing strategy will be employed such that the first 2 subjects (1:1, PIPE-505:Placebo) will be dosed and assessed for at least 24 hours prior to randomizing subsequent subjects (17:5, PIPE-505:Placebo). Dosing of the subsequent non-sentinel subjects may proceed after review and approval of the Day 1 safety and tolerability of the 2 sentinel subjects by the respective Investigator(s) at each site (as applicable) and Medical Monitor. If the Investigator(s) and Medical Monitor do not approve dosing of non-sentinel subjects, then the DMC will review the available data and provide further recommendations with respect to dosing of non-sentinel subjects.

3.3 Data Monitoring Committee

The DMC will be composed of at least one neuro-otologist and one audiologist, in addition to the Medical Monitor, a biostatistician, and Sponsor representatives. The DMC will be responsible for the following:

- Ongoing review of safety and tolerability of the study
- Review and analysis of all SAE reports
- Recommendation to re-initiate dosing if study suspension criteria were observed
- Recommendation to terminate the study at any point for safety purposes

Further details regarding the DMC, including membership, can be found in the DMC charter, which will be available prior to the administration of investigational product (IP). There is no planned interim analysis or adaptive design element in this study.

3.4 Study Suspension Rules

If any of the following occur, further dosing will be withheld until the events are evaluated by the DMC.

- Any 2 or more subjects experience the same or related severe AE, serious adverse event (SAE), or other medically significant event
- Any 2 or more subjects experience a 20 dB increase (i.e., worsening) in audiometric thresholds at any one test frequency (8000 Hz and below) or 10 dB increase at any two adjacent test frequencies at 30 days post-injection or beyond (8000 Hz and below) in the Index Ear.
- At the recommendation of the DMC based on ongoing review of cumulative safety data

Based on this assessment, the DMC will recommend to the Sponsor if the study should be terminated or continued, and/or whether implementation of additional safety monitoring is warranted. If relatedness to study drug cannot be refuted, then the events will be confirmed as dose limiting toxicity and dosing will not resume.

3.5 Number and Type of Subjects

The study is expected to enroll approximately 24 subjects with SNHL associated with speech-in-noise impairment. Specifically, the study subjects will demonstrate evidence of elevated hearing thresholds by audiometry and have difficulty hearing in the setting of background noise as measured by the QuickSIN test of speech-in-noise. It is estimated that between 60 and 80 subjects will be screened to complete enrollment.

While the placebo effect has historically been considered small in SNHL, including a blinded control group will delineate safety and tolerability issues related to the ROA and thermosensitive gel, as well as the potential treatment effect size. Given the modest to minimal placebo effect in this patient population, a 3:1 randomization was considered justified.

3.6 Sites and Regions

The study will be conducted in North America and is expected to enroll subjects across approximately 7 investigational centers in the US. Additional sites may be recruited to ensure timely enrollment of the appropriate subjects.

4. STUDY POPULATION

4.1 Inclusion Criteria

Subjects must meet each of the following inclusion criteria to be eligible for randomization:

1. Ability to personally provide written, signed, and dated informed consent to participate in the study.
2. Subject's primary language is English.
3. Male or female between 18 and 75 years of age, inclusive, at randomization.
4. Diagnosis of bilateral SNHL with air conduction threshold elevation by pure tone audiometry that is no greater than 55 dB at 250, 500, and 1000 Hz, no greater than 65 dB at 2000 Hz, and no greater than 85 dB at 4000 and 8000 Hz at Screening in either ear.
5. Threshold on the QuickSIN-EP > 4 dB SNR Loss at Screening in at least one ear.
6. Normal tympanogram in the ear intended for injection (i.e., the Index Ear), as guided by a range of -140 to +40 dekaPascals [daPa] and 0.3-2.9 millimhos [mmho]) at Screening, and as determined by the Investigator.
7. Male or female subjects with reproductive potential agree to comply with approved double barrier contraceptive method (e.g., condom plus intrauterine device [IUD], condom plus hormonal contraception, or double barrier method, i.e., condoms and diaphragms with spermicidal gel or foam) during and for 3 months after study drug administration.

   Subjects are considered of non-reproductive potential if:

   a. Post-menopausal female with ≥12 consecutive months of spontaneous amenorrhea and age ≥51 years with follicle-stimulating hormone (FSH) >30 mIU/mL at Screening
   b. Surgically sterile female and at least 6 weeks post-sterilization (i.e., bilateral oophorectomy or hysterectomy)
   c. Sterilized male at least 1-year post vasectomy and confirmed that they have obtained documentation of the absence of sperm in the ejaculate
8. The subject is in general good medical health with no clinically significant or relevant abnormalities, including medical history, physical exam, vital signs, electrocardiogram (ECG), and laboratory evaluations (hematology, chemistry, and urinalysis) as assessed by the Investigator.
9. An understanding, ability, and willingness to fully comply with study procedures and restrictions.

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met:

1. Montreal Cognitive Assessment (MoCA) score less than 26 at Screening.

2. History of chronic otitis externa or media, other chronic middle ear disorders, or perilymph fistula.
3. Presence of a genetic, syndromal or developmental auditory disorder.
4. Presence of an autoimmune or serious neurological disorder that could contribute to auditory loss, as determined by the Investigator.
5. History of herpes zoster oticus, or other infectious etiology of hearing loss as determined by the Investigator.
6. History of barotrauma as determined by the Investigator.
7. Evidence of current conductive hearing loss, mixed hearing loss or otosclerosis as determined by the Investigator, including significant air-bone gaps (i.e., >10 dB on audiometric testing, with the exception of 4000 Hz).
8. History of auditory loss related to exposure to known ototoxic drugs, such as high-dose aminoglycoside antibiotics (e.g., streptomycin, gentamicin, tobramycin, amikacin, neomycin, or minocycline), as determined by the Investigator.
9. Any prior exposure to platinum-based medications.
10. Otological disorders that would preclude safe tympanic injection (e.g., history or presence of cholesteatoma, TM perforation, otitis media, or middle ear anatomic anomaly) as determined by the Investigator.
11. Presence of a cochlear implant.
12. History of Meniere's disease or endolymphatic hydrops.
13. History of bothersome tinnitus, as determined by the Investigator.
14. Past otologic surgical procedures that would preclude safe intratympanic injection as determined by the Investigator.
15. Intratympanic injection within 6 months of randomization.
16. Use of an investigational product or intervention other than a non-interventional registry study (including vaccine studies) within the greater of 30 days or 5 half-lives (if known) prior to Screening or expected during the study.
17. Asymmetrical audiometric threshold results >15 dB between ears at any octave frequency between 250 and 8000 Hz at Screening AND the pattern of asymmetry is inconsistent with SNHL per Investigator judgment.
18. History of malignancy under current active treatment or considered at substantial risk for progression or recurrence during the study interval, as determined by the Investigator. Note, central nervous system neoplasms or head and neck cancer are excluded from eligibility regardless of treatment status.
19. The subject has a QTcF by ECG > 450 milliseconds for males or >470 milliseconds for females.
20. The subject has a history of dysrhythmia (such as atrial fibrillation or ventricular tachycardia, etc.) that is considered unstable by the Investigator.

[redacted]

21. The subject has experienced a significant systemic illness, as judged by the Investigator, within 30 days prior to Screening.
22. The subject has a current or relevant history of physical or psychiatric illness, or any medical disorder that may require treatment or make the subject unlikely to fully complete the study, or any condition that presents undue risk from the investigational product or procedure, as determined by the Investigator.
23. Known or suspected intolerance or hypersensitivity to the investigational product or closely-related compounds.
24. The subject is pregnant or breastfeeding or plans to become pregnant during the study.
25. History of alcohol or other substance abuse within 6 months of Screening as determined by the Investigator.
26. Subject is Hepatitis B positive (Subjects who are surface antibody positive secondary to immunization are eligible).
27. History of Hepatitis C.
28. Subject is Human Immunodeficiency Virus (HIV) seropositive.

4.3 Reproductive Potential and Pregnancy Avoidance

Sexually active females of childbearing potential (FOCP) must agree to use an acceptable form of contraception. FOCP will be advised to use acceptable contraceptives from the time of signing the Informed Consent Form (ICF) and for at least 3 months following the administration of the IP. If hormonal contraceptives are used they should be administered according to the package insert, in addition to use of an approved barrier method. FOCP who are not currently sexually active must agree to use acceptable contraception, as defined below, if they become sexually active during the study period.

Male subjects with reproductive potential agree to comply with approved double barrier contraceptive method from time of signing the ICF and for 3 months after IP administration. In addition, they must be advised to not donate sperm during this period.

An acceptable method of contraception is defined as one that has no higher than a 1% failure rate. In this study, the only acceptable methods of contraception are:

Condoms used with the following acceptable contraceptives:

- IUDs
- Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring)

Other acceptable double barrier contraceptive methods are:

- Double barrier methods (e.g., condoms and diaphragms with spermicidal gel or foam)
- Male condom PLUS spermicide
- Cap (plus spermicidal cream or jelly) PLUS male condom and spermicide

- Diaphragm (plus spermicidal cream or jelly) PLUS male condom and spermicide
- Copper T PLUS condom or spermicide.

4.4 Restrictions

Subjects should comply with the following restrictions after intratympanic injection.

- Prevent water exposure (e.g., shower spray) or submersion (e.g., swimming, bathing) of the injected ear, until cleared by follow-up otology examination to confirm closure of the TM opening. Subjects will be instructed on how to completely cover the Index Ear to enable showering until cleared.
- Abstain from heavy physical exertion for 24 hours following injection.
- No insertion of cotton-tipped applicators into the external ear canal of either ear until cleared by follow-up otology examination to confirm closure of the TM opening.
- No use of an occluding ear mold hearing aid in the Index Ear for 3 days following injection.

4.5 Withdrawal of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care; however, prior to randomization, it should be made clear to potential subjects that early withdrawal from the study including loss to follow up may pose some risk to the subject and can be damaging to the scientific research. Once a subject has been randomized, the Investigator will make every reasonable effort to keep the subject in the study.

The Investigator or Sponsor may withdraw the subject at any time during the study in the interest of subject safety. In such cases, the withdrawal of a subject by the Investigator should be discussed whenever possible with the Medical Monitor.

After study drug administration, subjects should continue to be followed through completion of the study unless a subject withdraws consent or is lost to follow up.

Regardless of the reason(s) for early termination of a subject, final evaluations are to be performed as completely as possible. Comments (spontaneous or elicited) or complaints made by the subject, the reason for termination, date of withdrawal, and the total amount of IP administered must be documented.

Subjects who discontinue prior to the Day 30 assessment for a reason other than an AE may be replaced and randomized to the same treatment assignment. Subjects who discontinue after Day 30 will not be replaced.

4.5.1 Reasons for Subject Discontinuation

Reasons for subject discontinuation include, but are not limited to:

- Adverse event: A subject may be discontinued from study due to AE only from the time of randomization (official study entry) until the time of IP administration. If a subject experiences an AE after the single dose IP administration, the subject should continue to be followed in the study and would only discontinue prematurely if the subject withdraws consent or is lost to follow up.
- Protocol violation or subject non-compliance.
- Subject withdrawal of consent: A subject should be considered to have withdrawn consent when the subject no longer wishes to participate in any aspect of the study, and does not want any further assessments, visits, or contact. A subject's refusal to participate in specific aspects of the study, such as a refusal to continue to provide blood samples, will NOT constitute withdrawal of consent. Investigators should make every effort to facilitate the subject's continued participation in remaining aspects of the study.
- Lost to follow-up: A subject will not be considered lost to follow-up until the end of the 12-week follow up period and after all efforts to contact the subject have been exhausted. At least 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). One of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations.

4.6 Early Termination of Study/Closure of Site

The study may be terminated early if new toxicological findings or results affecting the safety of the subjects become available. The Sponsor reserves the right to terminate the study at any time for any reason.

The Sponsor may terminate a study site at any time for any reason, including inadequate subject recruitment, Good Clinical Practice (GCP) non-compliance, poor quality data, evidence of attempted or proven fraud, or for any reason at the Sponsor's discretion.

In the event the Sponsor terminates a particular study site prior to the end of the trial, the site staff is responsible for notifying the Institutional Review Board (IRB).

5. PRIOR AND CONCOMITANT TREATMENT

5.1 Prior Treatment

Prior treatment includes all treatment (including herbal treatments, vitamins, non-pharmacological treatment such as psychotherapy as appropriate) received within 30 days of signing the ICF. Prior treatment information must be recorded on the appropriate case report form (CRF) page.

5.2 Concomitant Treatment

Concomitant treatment taken from the date of signing the ICF through the 90-day follow-up visit will be listed on the appropriate CRF page.

6. INVESTIGATIONAL PRODUCT

Refer to the PTI-505-101 Pharmacy Manual for further details regarding handling of the IPs in this study.

6.1 Description of Investigational Product

[redacted]

[redacted]

Additional information is provided in the IB and the Pharmacy Manual.

6.2 Randomization Code Generation and Storage

The randomization schedule will be generated by the Sponsor or designee and loaded to the Interactive Voice/Web Response System (IxRS) prior to the start of the study.

6.3 Administration of Investigational Product(s)

6.3.1 Index Ear Selection and Dosing Procedure

The IP or Placebo will be administered by an intratympanic injection as an out-patient procedure by a qualified otologist or neuro-otologist. The ear selected for dosing (i.e., the Index Ear) will be identified based on the audiometry and QuickSIN results at Screening as follows:

- The ear with the worse audiometry, as determined by the PI, will be identified as the Index Ear.
- If both ears are equal by audiometry, the ear with the highest SNR on the QuickSIN will be defined as the Index Ear.
- If the audiometry and QuickSIN are equal in each ear, the PI and subject will determine the Index Ear.

Otoscopy of the Index Ear canal and TM will be performed prior to administration and excessive earwax or debris will be manually cleared. At the discretion of the otologist or neuro-otologist performing the intra-tympanic injection, a topical anesthetic (e.g., EMLA cream [eutectic lidocaine/prilocaine mixture] or xylocaine [4% lidocaine solution or 10% pump spray]) may be applied to the external tympanic membrane. Given the transient discomfort associated with topical anesthesia and the extremely small gauge of the needle used for injection, the Investigator may also elect to perform the intra-tympanic injection without use of a topical anesthetic, after discussion with the subject. If applied, any remaining anesthetic in the external canal will be suctioned off prior to the injection in order to avoid spillage into the tympanic cavity and possible vertigo or dizziness. Any local anesthetic, if utilized, should be recorded in the subject's source record and the Concomitant Medications case report form.

The injection will be performed under otomicroscopy following standard transtympanic procedural technique, and with the subject reclining at an angle of up to 30° on the examination table or chair. The subject's head will be tilted 45° towards the unaffected ear during the injection and for 10 minutes following the administration. This will allow the round window niche and membrane to be at the lowest point of the tympanic cavity, facilitating the IP to collect in the round window niche and have direct contact with the round window membrane. The injection will be performed using a 1mL luer-lock syringe with a 3.5-inch 25-27-gauge needle (gauge based on Investigator preference). The posterior inferior quadrant of the TM will be punctured with the injection needle of choice, and the 200 microliters of either the PIPE-505 Suspension or Diluent alone (Placebo) will be delivered to the tympanic cavity over 5-10 seconds. The Investigator will maintain visualization of the needle bevel past the TM opening to avoid contact with the round window and other critical middle ear structures.

Subjects will remain in this position for approximately 10 minutes in order to avoid early loss of medication into the nasopharynx through the Eustachian tube. In addition, subjects will be asked to refrain from excessive swallowing, yawning, or speech for 30 minutes following the injection. After 30 minutes, the subject will have no restriction on activity, with the exception of restrictions outlined in Section 4.4.

6.3.2 Blinding the Treatment Assignment

Study drug supplies will be provided to sites as follows:

Open-label vials of Diluent for PIPE-505 Suspension (also administered alone as Placebo) will be provided in bulk format to be stored at 2-8 °C. An open-label Diluent vial will be utilized for each dose administration, whether mixed with the Active Ingredient or given alone as Placebo.

Identical appearing study drug kits will be supplied to sites and stored at controlled room temperature. Each kit will contain either an open-label vial of PIPE-505 Active Ingredient, or sham contents to mimic the weight of a vial of Active Ingredient. Kits will be labeled with a unique kit number to be assigned to subjects as they are randomized via the IxRS system for dosing. Although the trained pharmacist or nurse (i.e., dose preparer) preparing the study drug dose for administration is unblinded, the identical appearing blinded kits are utilized to prevent unblinding of blinded study staff who may have general access to the study drug storage areas.

An unblinded dose preparer will:

- Be responsible for preparing the study dose of PIPE-505 or Placebo according to the randomization assignment (kit number) obtained through the IxRS
- Deliver the preloaded syringe to the unblinded otologist or neuro-otologist responsible for administering the dose to the subject. The preloaded syringe will be covered to prevent content visualization by any other person (other than the responsible person performing the injection) Be identified on the site delegation of responsibility log as the unblinded dose preparer
- Be restricted from participating in any subject assessment
- Be restricted from disclosing treatment assignments to any study staff, with the exception outlined in Section 6.3.4

The Placebo formulation will consist of the thermosensitive gel (Poloxamer 407) alone. Due to formulation steps, as well as the clear color of the gel alone in comparison to the opacity of the PIPE-505 Suspension, the otologist performing the intratympanic injection cannot be masked from subject's assignment.

An unblinded otologist/neuro-otologist is responsible for study drug administration. The unblinded otologist or neuro-otologist is permitted to perform and/or interpret safety assessments including vital signs, electrocardiograms (ECG), clinical laboratory test results (routine hematology, chemistry and urinalysis), physical and otological examinations and AEs/SAEs as described in Section 8, Adverse and Serious Adverse Events Assessment. The unblinded otologist/neuro-otologist is prohibited from conducting audiometry, distortion product optoacoustic emission, auditory brainstem responses, electrocochleography (ECochG), middle ear muscle reflex, Speech Spatial Qualities of Hearing Scale, Word Recognition Score (WRS), Speech Recognition Threshold (SRT), Quick Sentences-in-Noise (QuickSIN), and Words-in-Noise (WIN).

The subject, all assessors responsible for evaluating the subjects or interpreting data obtained through these assessments, and other team members will remain blinded to subject assignment for the entire study duration (i.e., through database lock) with the exceptions of the dose preparer and otologist/neuro-otologist who administers study drug. The subject will be prevented from

visualizing the formulation during the procedure. All other Investigators and assessors will be blinded.

6.3.3 Allocation of Subjects to Treatment

The actual treatment administered to individual subjects is determined by the randomization schedule. Randomization will occur sequentially via the IxRS, in the order by which eligible subjects are randomized in the IxRS on Day 0 (Day 0 is the day of randomization and treatment). All subjects must have completed eligibility verification prior to treatment allocation.

The Subject ID number assigned at Screening will be retained throughout the study and used by the clinical site to identify the subject for all aspects of the study.

Overall, subjects will be randomized in a 3:1 ratio (PIPE-505:Placebo) but individually will be based on the following criteria:

- Sentinel subjects (i.e., first 2 subjects to be dosed) will be randomized in a 1:1 ratio (PIPE-505:Placebo)
- Remaining subjects will be randomized in a 17:5 ratio (PIPE-505:Placebo)

6.3.4 Unblinding the Treatment Assignment

The investigational drug blind shall not be broken unless information concerning the investigational drug is necessary for the medical treatment of the subject. All study assessments and causality assessment should be performed, if possible, prior to unblinding. In the event of a medical emergency, if possible, the Medical Monitor should be contacted before the investigational drug blind is broken to discuss the need for unblinding.

In the rare event that an emergency unblinding is required, the investigational drug treatment assignment can be obtained through the IxRS.

The Sponsor must be notified as soon as possible if the investigational drug blind is intentionally or accidentally broken. The date, time, and reason the blind is broken must be recorded in the source documents and the same information (except the time) must be recorded on the electronic case report form (eCRF).

Unintentional unblinding of treatment assignment must be reported to the Sponsor as soon as possible, and within 24 hours of occurrence. Subjects who are unintentionally unblinded (i.e., subject or blinded assessor becomes aware of their treatment assignment for reasons other than medical emergencies) will continue in the study. An additional subject may be added at the discretion of the Sponsor, in consult with the DMC. A blinded site staff member who becomes unblinded for a given subject will be prohibited from subsequent audiological assessments of that subject and will need to be replaced.

No change should be made to any safety assessment of the subject after unblinding.

Following assessment of the AE data and pre-defined criteria for study termination, dosing may be interrupted/stopped and the blind broken for further analysis. Based on review of unblinded data, the Sponsor in consultation with the DMC and Investigators will decide if and how it is appropriate for the study to proceed.

6.4 Labeling, Packaging, Storage, and Handling

6.4.1 Labeling

Labels pertaining to the investigational supply vials must meet all applicable requirements of the FDA, Annex 13 of cGMP (Manufacture of Investigational Medicinal Products, July 2000) and/or other local regulations, as applicable.

6.4.2 Packaging

[redacted]

6.4.3 Storage

All IP kits and Diluent vials will be stored as per the labeled instructions.

The Investigator has overall accountability for ensuring that IP is stored in a secure, limited-access location. Dose preparation should be delegated to the trained unblinded pharmacist or nurse and must be documented. IPs are formulated and transported to the individual responsible for administration in a blinded manner.

IP must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage locations to ensure that the IP is maintained within an established temperature range. The Investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device, such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (e.g., certified Min/Max Thermometer) would require manual resetting upon each recording. The Sponsor must be notified upon discovery of any excursion from the established range. Temperature excursions will require site investigation as to cause and remediation. The Sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should product be dispensed to subjects until the impact is determined and product is deemed appropriate for use by Sponsor.

6.4.4 Handling

Detailed dose preparation instructions are provided in the Pharmacy Manual. Individual doses in the syringe prepared for administration to a subject should be covered by a cooling blanket or pad from the time of formulation until administration, protected from light, and must be administered within 3 hours of formulation.

To maintain the blind, doses prepared for administration will be delivered in a blinded manner from the dose preparer to the investigator who will administer the dose. Neither the study subject, nor any other blinded staff should be permitted to visualize the content. Once prepared, the dose will be masked (e.g., sealed box or other type of secure cover) during transport and until administration to prevent unblinding of any blinded staff members.

6.5 Investigational Product Quality Complaints

Investigators are required to report IP quality complaints to the Sponsor within 1 business day. This includes any instances wherein the quality or performance of the IP does not meet expectations (e.g., inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (e.g., wrong product such that the label and contents are different products). Reporting instructions are included in the Pharmacy Manual.

6.6 Drug Accountability

Investigators will be provided with sufficient amounts of the blinded IP kits to carry out this protocol for the agreed number of subjects. The receiving study staff will acknowledge receipt of all study drug kit shipments via the IxRS system. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The Investigator has overall responsibility for ensuring that an appropriate delegate, i.e., unblinded trained pharmacist or nurse, prepares and dispenses IP for the appropriate subjects per the study protocol and Pharmacy Manual. This delegation must be documented in the applicable study delegation of authority form.

The Investigator is also responsible for ensuring that the IP is administered only to subjects included in this study by a qualified designee (i.e., the unblinded otologist, as documented by the Investigator in the applicable study delegation of authority form) following the procedures set out in the study protocol and Pharmacy Manual. Each subject will be given only the IP carrying his/her treatment assignment. All dispensing will be documented in the IP accountability records.

After the tear-off label has been removed from the assigned study drug kit and affixed to the Drug Accountability Log, the unblinded dose preparer and/or administering otologist/neuro-otologist should properly dispose used kits, vials, needles, and syringes immediately upon use per local, state, and national regulations.

No IP stock or returned inventory from the Sponsor may be removed from the site where originally shipped without prior knowledge and consent by the Sponsor. If such transfer is authorized by the Sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The Sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records provided that the overall blind of the study is not compromised.

At the end of the study, or as instructed by the Sponsor, all unused stock and remaining unused IP packaging may be sent to the Sponsor or designee. IP being returned to the Sponsor or designee must be verified by the Sponsor or designee prior to return shipment. Tamper evident features must not be broken. Shipment returns must be documented on the appropriate accountability records. Shipment of all returned IP must comply with local, state, and national laws.

With the written agreement of the Sponsor, unused stock and empty/used IP packaging may be destroyed at the site or a local facility according to the site's standard operating procedures (SOP). In this case, destruction records must be retained with copies provided to the Sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Based on entries in the site drug accountability forms, it must be possible to reconcile IPs delivered with those used and returned. All IPs must be accounted for and all discrepancies investigated and documented.

7. STUDY PLAN

7.1 Study Schedule

All procedures will be conducted according to the SOE provided in Table 2. For any given procedure, subjects are to be assessed by the same Investigator or site personnel, whenever possible, throughout the duration of the study.

7.1.1 Screening Period

Screening assessments may begin after the subject has provided written informed consent as documented by a signed ICF. Screening procedures may be conducted over multiple days but must occur within 56 days of randomization.

7.1.1.1 Subject ID Number Assignment & Tracking

Upon signing of an ICF, each screened subject will be registered for Screening in the IxRS and assigned a unique Subject ID number to be retained throughout the duration of the study. The subject ID number will consist of 6 digits (XXX-YYY; where XXX = 3-digit site number and YYY = 3-digit sequential number within each site).

7.1.1.2 Screen Failures

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or meets at least 1 of the exclusion criteria and has not been randomized or administered IP as defined by the protocol.

Screening assessments may be repeated by Investigator discretion if the Investigator believes the initial assessment may not be correct or an anomaly. Screen failed subjects may be rescreened for the study one time if the Investigator believes that circumstances have changed such that the subject would become eligible and the Medical Monitor agrees. Re-screened subjects are registered in IxRS as new screens and receive a new Subject ID number. Subject ID numbers assigned to subjects who fail Screening are not reused.

Investigators must account for all subjects who sign the ICF. Reasons for screen failure must be documented in the subject record and eCRF for all screen failed subjects.

The primary reason for screen failure is recorded using the following categories:

- Did not meet inclusion criteria or did meet exclusion criteria
- Significant protocol deviation
- Lost to follow-up
- Voluntary withdrawal
- Study termination
- Other

7.1.2 Randomization and Dose Administration Visit (Day 0)

Subjects who qualify for the study will return to the study clinic for additional baseline and pre-dose procedures and confirmation of continued eligibility as per the SOE. Following randomization and administration of the study dose, subjects will remain in the clinic until completion of the assessments at 30 minutes post-injection, after which the subjects may leave the clinic but must return to undergo the 4-hour post-injection assessments as per the SOE. Subjects may be kept in the clinic longer after either post-injection assessment based on the Investigator's medical discretion (e.g., to manage any AEs).

7.1.2.1 Interactive Voice/Web Response System for Subject Tracking/Data Collection

An IxRS will be used to assign randomized treatment assignments. Instructions for use of the IxRS will provided in a separate manual.

7.1.3 Follow-up Period (Days 1-90)

Subjects will return to the study clinic in accordance with the SOE (Table 2) for follow-up procedures over the course of 3 months, starting 1 day after the study drug administration.

7.1.4 Early Termination

Subjects who discontinue early will be encouraged to return to the site to complete early termination assessments for safety purposes. Early termination procedures should follow the Day 90 schedule according to the SOE (Table 2). The reason for discontinuation must be documented in the source document and eCRF.

For all subjects receiving study medication, the Investigator must complete the End of Study eCRF page.

7.1.5 Additional Care of Subjects After the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

Note, detailed descriptions of the methods used for the audiological assessments (Section 7.2.10 – Section 7.2.22 below) are included separately in the Study Assessment Manual.

7.2.1 Informed Consent

The requirements of the ICF are described in Section 10.3.1. The ICF must be obtained prior to the subject entering into the study, and before any protocol-related procedures are performed, including requesting that a subject fast for laboratory evaluations.

7.2.2 Demographics, Medical History, and Medication History

Demographic information to be obtained will include date of birth, sex, Hispanic ethnicity, race as described by the subject, and smoking status of the subject at Screening.

Medical history to be obtained will include determining whether the subject has any significant conditions or diseases that stopped at or prior to signing the ICF. The medical history should also include a detailed audiological interview.

Medication history information to be obtained includes any medication relevant to eligibility criteria stopped at or within 30 days prior to signing of ICF.

7.2.3 Physical Examination

Complete physical examinations per each institution's guidelines, will be performed according to the SOE (Table 2). Body weight will be measured at Screening and Visit 7 (End of Study), and height will only be measured at Screening. Abnormalities identified at the Screening Visit will be documented in the subject's source documents and on the medical history CRF.

Any abnormal change from the baseline physical examination assessment must be assessed as not clinically significant or clinically significant by the Investigator and recorded in the source document and eCRF. Any clinically significant change or new diagnosis as a result of a clinically significant change, as determined by the Investigator, will be recorded as an AE in source documentation as described in Section 8.

7.2.4 Otological Examination

The otologic examination will include direct examination of the external ear, ear canal and TM by otoscope, as well as standard testing of tympanic mobility. The otological examinations will be conducted by the unblinded otologist or neuro-otologist who performs the intratympanic injection and also conducts all post-injection otological examinations through at least Day 30, and preferably throughout the study.

7.2.5 Vital Signs

Vital signs (pulse rate, respiration rate, body temperature, and blood pressure [BP]) will be recorded after the subject has been seated or supine for at least 5 minutes.

On Day 0 (Dosing Day), vital signs are conducted at 3 time points: pre-dose, 30 (±5) minutes post-dose, and 4 hours (±15 minutes) post-dose.

BP should be determined by cuff (using the same method, the same arm, and in the same position throughout the study). Any clinically significant deviations from baseline vital signs which are deemed clinically significant in the opinion of the Investigator are recorded as an AE.

Vital signs should be measured at the same time of the day across visits if possible. When vital signs are scheduled at the same visit as blood draws, the vital signs should be recorded first.

7.2.6 ECG Procedure

Standard 12-lead ECGs will be recorded at timepoints specified in the SOE (Table 2). The ECG at Screening may be repeated at the Investigator's discretion to confirm the QTcF result. The Day 0 ECG will only be conducted at 4 hours (±15 minutes) post-dose. Subjects should be in a supine position following an approximate 5-minute rest period for ECG recordings. When an ECG is scheduled at the same time as blood draws or vital signs, the ECG should precede the vitals and blood draw. Additional unscheduled ECGs may be recorded where clinically necessary for subject safety.

All stationary 12-lead ECG machines will be capable of measuring QTc. Should technical difficulties occur during recording of the ECG, a reasonable attempt should be made to repeat the ECG shortly after the failed attempt. One copy of the 12-lead ECG with the physician's signature and date of assessment will be filed with the source documents.

7.2.7 Pregnancy Testing

FSH levels will be obtained on post-menopausal women only to verify childbearing potential.

A serum beta human chorionic gonadotropin (HCG) pregnancy test is performed on all females of child-bearing potential (FOCP) during the Screening Period. A urine pregnancy test will be performed at subsequent timepoints. If pregnancy is suspected prior to dosing, the subject will be withdrawn from the study. If pregnancy is suspected after dosing, the subject should remain in the study for follow-up safety procedures through study completion.

7.2.8 Montreal Cognitive Assessment (MoCA)

The MoCA is a brief 30-question test that assesses subjects for dementia. The test evaluates orientation, short-term memory, executive function, visuospatial ability, language skill, attention, and abstraction. Scores on the MoCA range from zero to 30, with a score of 26 or higher generally considered normal. The test takes 10-12 minutes to administer.

7.2.9 Laboratory and Auditory Evaluations

7.2.9.1 Clinical Safety Laboratory Assessments

A central lab, selected and contracted by the Sponsor, will be responsible for analysis of laboratory tests outlined in Table 3. The central lab will provide a lab manual with sample processing instructions, sampling kits and shipping materials to each site. The name and address of each clinical laboratory used in this study will be maintained in the Investigator's files at each site.

All clinical laboratory assays will be performed according to the laboratory's normal procedures. Reference ranges supplied by the laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The Investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Abnormal clinically significant laboratory values, which are unexpected or not explained by the subject's clinical condition may be, at the discretion of the Investigator or Sponsor, repeated until confirmed, explained, or resolved.

Clinical laboratory tests are listed in Table 3.

Table 3: Clinical Laboratory Tests

Hematology	Serum Chemistry	Urinalysis[1]	Coagulation[1]	Other tests[1]
Hematocrit Hemoglobin Platelets Red blood cell (RBC) count White blood cell (WBC) w/ differential (% and absolute)	alanine aminotransferase albumin alkaline phosphatase amylase aspartate aminotransferase bicarbonate blood urea nitrogen calcium chloride creatine kinase creatinine gamma-glutamyl transferase glucose inorganic phosphorus lactate dehydrogenase lipase potassium sodium total bilirubin total protein uric acid	Bilirubin Blood Glucose Ketones leukocyte esterase microscopy[5] nitrite pH protein specific gravity urobilinogen	activated partial thromboplastin time international normalized ratio partial thromboplastin time	Serum Pregnancy[2] Urine pregnancy[3] FSH[4] Hepatitis B Hepatitis C HIV

[1]performed only at Screening
[2]performed only in FOCBP at Screening
[3]performed only in FOCBP on Day 0 prior to Randomization and at either Early Termination or Completion
[4]performed only in post-menopausal women
[5]performed only as needed based on positive dipstick test results

[redacted]

7.2.9.2 Blood Sampling for PK Analysis

Blood samples for analysis of PIPE-505 plasma concentrations will be collected at each of the designated timepoints. Note that on Day 0, PK samples will be collected pre-dose and 4 hours (±15 minutes) post-dose. Instructions for sample collection, processing, storage, and shipment are provided in a separate lab manual. All plasma PK samples will be analyzed by a central bioanalytical lab.

7.2.10 Tympanometry

Tympanometry assesses the relationship between the air pressure in the ear canal and the movement of the TM. The test measures the compliance of the TM to changes in air pressure, as well as reflex contraction of the middle ear muscles (Section 7.2.11).

Otoscopic examination will document that the TM is clear and without perforation. A tympanometer probe is inserted into the ear which changes pressure within the canal and generates a tone of 226 Hz. The generated tone is transmitted through the middle ear and the reflected sound is expressed as compliance. The data measured by tympanometry are plotted as a tympanogram that characterizes the ear canal volume (cm^3), max pressure (daPa), and peak compliance (mL). A Type A tympanogram is considered reflective of normal pressure in the middle ear and normal mobility of the TM and ossicles. Proof of calibration of the tympanometer will be documented for each site.

7.2.11 Middle Ear Muscle Reflex (MEMR)

The MEMR (also referred to as the stapedius or acoustic reflex) measures the function and integrity of the middle and inner ear, as well as the seventh and eighth cranial nerves (proximal to the innervation of stapedius). The MEMR is used to monitor the stapedial reflex that occurs in response to high-intensity sound, and employs the same technique as used with tympanometry. When presented with a high-intensity sound stimulus, the stapedius and tensor tympani muscles of the ossicles contract. The stapedius stiffens the ossicular chain by pulling the stapes of the middle ear away from the oval window of the cochlea and the tensor tympani muscle stiffens the ossicular chain by loading the TM when it pulls the malleus in toward the middle ear. The reflex decreases the transmission of vibrational energy to the cochlea.

The MEMR measures the sound pressure level from which a sound stimulus of a given frequency triggers the acoustic reflex (i.e., when the stapedius muscle contracts, the ossicular chain stiffens, causing a small change in compliance in the middle ear system that is detected by the tympanometer). Dynamic changes which result from contraction of stapedius in response to stimuli of 1000, 2000, and 4000 Hz, at intensities of 70–115 dB sound pressure level, are measured and the thresholds for activation documented. Both ipsilateral and contralateral stimulation will be [redacted]mployed.

[redacted]

7.2.12 Speech Recognition Threshold (SRT)

The SRT is the softest intensity spondee word (tested in quiet conditions) that the subject can repeat at least 50% of the time. Spondees are defined as bisyllabic words that equally emphasize both syllables. The bisyllabic words are presented to each ear separately through insert earphones in the sound booth. Based on the pure tone thresholds, the initial intensity is set at an audible level. The subject is asked to repeat the presented word. If the response is correct, the stimulus intensity if decreased by 10dB; an incorrect response will lead to an increase in the stimulus intensity by 5dB. Standard bracketing procedures are continued until the stimulation level for speech recognition is 50% correct, thereby identifying the SRT for that subject. Effective masking should be provided (and recorded) as per the audiologist conducting the test. The SRT result for each ear should be consistent with the pure tone average.

7.2.13 Word Recognition Score (WRS)

The WRS in quiet assesses the ability to repeat phonemically balanced single syllable words unknown to the subject. The words are presented at 35-40dB SL or at Most Comfortable Level (MCL), as determined by the examiner, via insert earphones or headphones in the sound booth. The recorded list of NU-6 words (Northwestern University Auditory Test No. 6) are presented to each ear separately, and the subject is asked to repeat the words. The number of correct and incorrect responses are documented as a percentage for each ear.

7.2.14 Tinnitus Reaction Questionnaire (TRQ)

The TRQ is a self-reported scale designed to assess the psychological distress associated with tinnitus. The scale records tinnitus-related effects on lifestyle and general well-being. Specifically, the 26-item scale covers general distress, interference with work and leisure activities, signs of severe distress, and avoidance of activities. The subject rates each item on a 5-point scale according to the degree of applicability.

7.2.15 Speech, Spatial, and Qualities of Hearing Scale (SSQ)

The SSQ was developed to assess listeners' subjective sense of listening ability and listening experience in everyday complex situations that often involve spatial hearing. The 49-item self-assessment of hearing measures the ability/disability in a variety of complex listening situations typical of those encountered in everyday life. The subject will complete the response to each question and the answers will be scored by the Investigator.

7.2.16 QuickSIN-EP

The QuickSIN™ (Etymotic Research) is a speech-in-noise test that quickly and easily measures the ability to hear in noise. The Quick SIN includes a total of 18 unique 6-sentence lists composed of Institute of Electrical and Electronics Engineers (IEEE) sentences, which were designed to provide limited contextual cues to aid in understanding. The sentences are spoken by a female talker and are presented at a constant level in a background of 4-talker babble (one male and three females). The background noise is considered most representative of the noise encountered in

typical social situations. The babble level in each list decreases in intensity in 5 dB steps from +25 to 0 dB in order to vary the signal-to-noise ratio (SNR) (Killion et al., 2004). The test provides an assessment of the subject's ability to understand speech in noise as a SNR rather than as a percent correct score. For the purposes of this study, the QuickSIN performed with insert earphones will be referred as QuickSIN-EP.

The subject is asked to repeat the sentences presented by insert earphones in the sound booth, and each sentence has 5 key words. Each correctly repeated word is awarded one point for a possible score of 30 points per list. The score is determined by the use of the formula 25.5 - Total Words Correct = SNR loss. The SNR loss score, calculated for each ear separately, represents the SNR a listener with hearing loss requires above the SNR a normal hearing listener requires to achieve 50% correct sentence identification (Killion, 1997). The test will be conducted according to the most current manual and version.

The QuickSIN will also administered by sound field (see Section 7.2.18 below) to assess binaural speech-in-noise performance.

7.2.17 Words-in-Noise (WIN)

The Words-in-Noise test evaluates the ability of a listener to understand words in the setting of multi-talker babble background. The level of the multi-talker background is fixed and the stimulus words are presented at seven signal-to-noise ratios from 24 to 0dB in 4 dB decrements. The word lists are based on the Northwestern University Auditory Test No. 6 (Tillman and Carhart, 1966) and spoken by a female speaker. The monosyllabic words are pre-recorded with a noisy background. The test is administered using insert earphones in the sound booth and is conducted in each ear separately. The test assesses the signal-to-noise ratio for each ear at which recognition performance is 50% according to the Spearman-Karber equation (Wilson et al., 2007).

7.2.18 Sound Field Speech Test: QuickSIN-SF

Sound-field (free-field) Speech testing signals are presented via a single front speaker at a 0° azimuth to the subject's face who is positioned in the center of the room. The QuickSIN test content will be used to assess speech detection in the setting of background multi-talker babble, allowing binaural measurement of speech-in-noise performance in the sound field. For the purposes of this study, the QuickSIN performed in the sound field will be referred as QuickSIN-SF.

7.2.19 Pure Tone Audiometry

The pure tone audiogram measures the subject's hearing ability by frequency (pitch) and intensity (volume) by both air and bone conduction. The audiogram determines the degree, type, and configuration of hearing loss. The test assesses air and bone conduction thresholds for each ear over frequencies from 250Hz to 8000Hz (note, bone conduction is not measured at 250 or 8000Hz). Extended high frequency hearing from 10000Hz to 16000Hz will also be assessed, if available based on site audiometer specifications. To reduce ambient noise, pure-tone audiometry will be performed in a sound booth connected to an external audiometer with insert earphones up to 8000Hz and circumaural headphones for frequencies above 8000Hz. The insert earphones are

small transducers which fit into the ear canal and further reduce ambient noise and crossover of auditory stimuli to the non-test ear.

The audiometry, using pulsed tone stimulus, will be performed according to standards as outlined by the modified Hughson Westlake procedure as per ANSI standards (ANSI S3.21-1978 [R-1982]). Pure tone air and bone conduction threshold audiometry may be measured with and without masking. Effective masking levels should be recorded. An audiometer instrument/device typical to audiology clinics, including high frequency capabilities when available, will be used to present pulsed pure tones and determine thresholds. For thresholds of frequencies between 0.25 and 8 kHz (0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) insert earphones (ER-3A) will be placed into the ear canal. To determine ultra-high frequency thresholds at 10, 11.2, 12.5, 14, 15, and 16 kHz, circumaural earphones will be used, if available based on site audiometer specifications. A modified Hughson-Westlake procedure will be used to determine thresholds at each frequency using a down 10 dB, up 5 dB step size for intensity and where threshold is the lowest pulsed tone intensity that the subject responds at 50% accuracy in at least 2/3 trials.

Documentation confirming the annual standardized calibration of the audiometer, earphones, and speakers will be required.

7.2.20 Distortion Product Otoacoustic Emission (DPOAE)

The DPOAEs are an objective indicator of normally functioning cochlear outer hair cells. DPOAE are generated by the OHCs as they expand and contract in response to two simultaneous tones of different frequency. The test does not require a response from the subject, and a sound booth is not required.

Subjects will be fitted with a soft flexible probe, consisting of a sensitive microphone and transducers, which will create a seal when inserted into the ear canal. DPOAEs will be tested in at least 4 frequencies with a single stimulus level and varying frequency. To be considered a present DPOAE, the noise floor will be assessed to determine if it is low enough to evaluate the responses and the difference between the response and the noise floor at each frequency will exceed 5 dB.

Documentation confirming the annual standardized device calibration will be required.

7.2.21 Auditory Brainstem Response (ABR)

ABR audiometry is a neurologic test of auditory brainstem function in response to brief auditory (e.g., click or tone-burst) stimuli. Auditory evoked potentials are extracted from underlying brainstem electrical activity and recorded by electrodes placed on the scalp or in the ear-canal with the subject in the sound booth or a quiet room.

The normal response to the 2-4 kHz click stimulus presented at high intensities (e.g., 70-90 dB normalized hearing level [nHL]) consists of a series of neutrally-generated waveforms within 10 milliseconds of the auditory stimulus by tip-trode. Waves I-III are generated by the auditory branch of the eighth cranial nerve, and Wave IV-V is generated by the upper brainstem. The ABR waveforms will be assessed by amplitude (microvoltage), latency (milliseconds), interpeak latency, and interaural latency.

The ABR Wave I response is a far-field representation of the compound auditory nerve action potential (AP) in the distal portion of the 8th cranial nerve and is hypothesized to arise from the dendrites of the auditory nerve. It has been suggested that ABR Wave I amplitude, as well as the summating potential (SP)-to-AP ratio and speech recognition in noise, offers an effective nonbehavioral measure of CS (Barbee et al., 2018). Transducer calibration will be documented at each site.

7.2.22 Electrocochleography (ECochG)

ECochG is similar to ABR in that electrical potentials are measured in response to sound stimulation but is recorded using a non-invasive extra-tympanic electrode placed in the ear canal (i.e., tip-trode). The three potentials generated by ECochG include the cochlear microphonic, the SP and AP. The SP:AP ratio is considered possibly related to the status of the cochlear synapse.

The ECochG will be conducted as a component of the ABR. The ipsilateral electrode will be paired with the contralateral, or reference, electrode in the non-test ear. The forehead or nasion will be used as the ground electrode. Impedance should be less than 5000 ohms, but may be higher for the ipsilateral electrode. The transducer signal will consist of a click at 90 dB nHL, and the test will be repeated until a reliable waveform is observed. Transducer calibration will be documented [redacted] each site.

[redacted]

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An ***Adverse Event*** **(AE)** is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that 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 related to the medicinal (investigational) product (ICH Guidance E2A,1995).

All AEs should be captured on the AE CRF.

All AEs are collected from the time the informed consent is signed until the final visit is completed at approximately 90 days post-injection. All AEs are to be recorded on the appropriate AE pages in the CRF and in source documents. Where possible, a diagnosis, rather than a list of symptoms, should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE CRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), an outcome is reached, stabilization (the Investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained regardless of whether the subject is still participating in the study. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of IP, but the dyspepsia becomes severe and more frequent after first dose of IP has been administered, a new AE of severe dyspepsia [with the appropriate date of onset] is recorded on the appropriate CRF). The medical assessment of severity is determined by using the definitions provided in Table 4:

Table 4: Adverse Event Severity

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

8.1.2 Relationship Categorization

The Investigator, whether blinded or unblinded (i.e., the otologist or neuro-otologist performing the intratympanic injection), must evaluate the potential relationship to "Study Drug" for each AE, wherein "Study Drug" will include both Placebo and PIPE-505 Suspension. Therefore, the Investigator should attribute causation to the Study Drug if the AE is most consistent with an effect secondary to injection of a substance, either Placebo or PIPE-505 Suspension, into the tympanic space. If there is no valid reason for suggesting a relationship, then the AE should be classified as 'not related'. Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the 'Study Drug' and the occurrence of the AE, then the AE should be considered 'related'. The causality assessment in relation to Study Drug (without recording any unblinding information) must be documented in the source document and graded according to Table 5 below. The AE patterns specific to treatment assignment (subjects who received active PIPE-505 Suspension versus Placebo) will ultimately be determined upon final analysis of the unmasked database at study conclusion.

Table 5: Adverse Event Causality Assessment

Term	Relationship	Definition
Related	Yes	The temporal relationship between the event and the administration of the investigational product is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	No	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the investigational product and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the CRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved with Sequelae
- Recovering/Resolving
- Unknown

8.1.4 Symptoms of the Disease Under Study

Subjects with SNHL associated with speech-in-noise impairment may experience the onset of tinnitus, hyperacusis, and reduced hearing capability consistent with the spectrum hearing loss symptomatic progression.

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected progression of the disease. However, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory Evaluations

A change in the value of a clinical laboratory investigation can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory values which were not present at baseline, further clinical or laboratory investigations should be performed until the values return to within the reference range, the value stabilizes, or until a plausible explanation (e.g., concomitant disease) is found for the abnormal clinical laboratory values.

The Investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory parameter is clinically significant and therefore represents an AE.

8.1.6 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the final visit is completed at approximately 90 days post-injection. Any report of pregnancy for any female subject

or the partner of a male subject must be reported within 1 business day to the Sponsor (Table 1) using the Pregnancy Report Form. Since this is a single dose study, no further dosing will be administered to the subject and the subject will continue to be followed according to the SOE, unless, at the discretion of the Investigator, specific tests are contraindicated for the pregnancy.

If the female subject and/or female partner of a male subject agrees to the primary care physician being informed, the Investigator should notify the primary care physician that the subject/female partner of the subject was participating in a clinical study at the time she became pregnant and provide details of treatment the subject received.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the Investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post-partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the SAE Form. Note, an elective abortion is not considered an SAE.

8.1.7 Medication Error

Medication error (as defined below) must be reported to the Sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described previously in this section. Note: The 1 business day reporting requirement for SAEs does not apply to reports of medication errors unless these result in an SAE.

- **Medication Error** – An error made in prescribing, dispensing, administration, and/or use of an IP.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the IB which the Sponsor has provided under separate cover to all Investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the Investigator to the Pharmacovigilance contact within 1 business day of the first awareness of the event. Note: The 1 business day reporting requirement for SAEs does not apply to reports of medication errors (see Section 8.1.7) unless they result in an SAE.

The Investigator must complete, sign, and date the SAE Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Pharmacovigilance Department (Table 1).

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event*** **(SAE)** is any untoward medical occurrence (whether considered to be related to IP or not) that at any dose:

- Results in death
- Is life-threatening

  NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Is an important medical event; Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.

Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE.

However, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meets serious criteria must be reported as SAE(s).

8.2.4 Serious Adverse Event Collection Timeframe

All SAEs are collected from the time the subject signs informed consent until the final study visit at approximately 90 days post-injection. All SAEs occurring during this period must be reported to the Pharmacovigilance contact (Table 1) within 1 business day of the first awareness of the event.

8.2.5 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject prior to study entry or leading up to the onset date of the SAE or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.6 Fatal Outcome

Any SAE that results in the subject's death (i.e., the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received IP).

8.2.7 Regulatory Agency, Institutional Review Board, Independent Ethics Committee, and Site Reporting

The Sponsor is responsible for notifying the relevant regulatory authorities and the Contract Research Organization (CRO) is responsible for notifying study sites and the central IRB of related, unexpected SAEs.

In addition, Sponsor is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the PIPE-505 program.

The Investigator is responsible for notifying his/her local IRB or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

An eCRF for each subject will be provided. All appropriate subject data gathered during the study will be recorded in English on these forms. Whenever possible, all information requested on the eCRF should be completed. If information is not available, it should be documented as such.

9.2 Clinical Data Management

Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and/or manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

The completed CRFs or completed print out and/or electronic copy of the eCRFs for this study are the property of Pipeline and should not be made available to third parties, except for authorized representatives of appropriate health/regulatory authorities, without written permission from Pipeline.

9.3 Statistical Analysis Process

Details regarding the statistical methods and definitions will be provided in the Statistical Analysis Plan (SAP). The SAP will serve as a complement to the protocol and supersedes it in case of differences. The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

All statistical analyses will be performed after the database is locked and unblinded. Statistical analyses will be performed using Version 9.4 or higher of SAS® (SAS Institute, Cary, NC 27513). Summary tables and listings of data will be provided. Summary statistics will be presented by dose group and for all subjects combined as appropriate. For continuous variables, data will be summarized with the number of subjects (N), mean, standard deviation, median, minimum, and maximum by treatment group. For categorical variables, data will be tabulated with the number and proportion of subjects for each category by treatment group.

9.4 Selection of Subjects to be Included in the Analyses

Enrolled Set – Subjects who have signed informed consent and have begun some study procedures (e.g., dispensed IP current drug has been withdrawn).

Randomized Set – Enrolled subjects for whom a randomization number has been assigned.

Safety Analysis Set – Enrolled/Randomized subjects who have undergone dosing of IP. Safety data for these subjects will be summarized by treatment actually received.

Full Analysis Set – Subjects in the Safety Analysis Set who have at least 1 post-baseline value efficacy assessment. Efficacy data will be summarized by assigned treatment regardless of actual treatment taken.

Per-Protocol Set – Subjects in the Full Analysis Set who do not have pre-defined protocol deviations and who completed the final scheduled assessment.

Pharmacokinetic Set – Subjects in the Safety Analysis Set with no major deviations related to IP intake, for whom the primary PK data is considered sufficient and interpretable.

9.5 Subject Disposition

Disposition of subjects in each analysis set will be summarized by treatment group using descriptive statistics. In addition, for subjects who prematurely discontinue from the study, the reasons for discontinuation will be summarized by treatment group.

9.6 Demographic and Baseline Characteristics

Descriptive summaries of demographic and baseline characteristics will be presented by treatment group and overall for the Safety Analysis Set and Full Analysis Set (FAS).

Demographics and baseline characteristics will be examined to assess the comparability of the treatment groups at baseline. Continuous variables such as subject age, weight, height, and body mass index (BMI) will be summarized using number of observations, mean, standard deviation, median, minimum, and maximum values. Categorical variables such as subject sex and race will be summarized using number of observations and percentages for each category.

Medical history and history of SNHL will be summarized by treatment group using the number of observations and percentages of subjects reporting each category.

9.7 Prior and Concomitant Medication

Prior and concomitant medications will be coded using the most current version of the WHO drug dictionary, as of the start of the study. Prior and concomitant medications will be listed and summarized by therapeutic area preferred drug name and treatment group.

9.8 Safety Analyses

AEs will be coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA) as of the start of the study. The number of events, incidence, and percentage of treatment-emergent adverse events (TEAE) will be calculated overall, by system organ class (SOC), and by preferred term. Events with missing onset dates will be included as treatment-emergent. Treatment emergent events are considered those events with onset dates on or after

study drug exposure or events with dates of onset or worsening after study drug exposure. If a subject experiences more than one occurrence of the same AE, the occurrence with the greatest severity and the closest association with the study drug will be summarized. TEAEs will be further summarized by severity and relationship to IP. AEs related to IP, AEs leading to withdrawal, SAEs, and deaths will be summarized/listed.

Clinical laboratory tests, vital signs, and ECG findings will be summarized by treatment group and visit. Change from baseline values will be summarized using shift tables. Potentially clinically important findings will also be summarized or listed.

9.9 Preliminary Efficacy Analyses

As described in Section 2.2.3, efficacy endpoints include mean change from baseline to Month 3 post-baseline in:

- WRS (performed in quiet)
- QuickSIN-EP and QuickSIN-SF
- SSQ
- SRT
- WIN
- Pure tone audiometry
- DPOAE
- MEMR
- ABR
- ECochG.

Efficacy parameters will be summarized and analyzed separately for the Index Ear and Non-Index Ear using the FAS as the primary population for efficacy analyses. In addition, differences between treatment groups will be calculated as study drug minus Placebo. Change from baseline will be calculated as the value at a follow-up study visit minus the baseline value, where baseline value is defined as the last non-missing measurement recorded prior to initiation of study drug. Summaries of mean change from baseline for each efficacy data will be presented by treatment group and by study visit, as appropriate, with associated 95% confidence intervals.

Exploratory inferential analyses will be conducted to compare efficacy outcomes in subjects assigned to study drug versus those assigned to Placebo. In addition, comparisons of each dose of test drug to Placebo will be conducted. The null hypothesis to be tested is that there is no difference for the efficacy variable between test drug and Placebo with the alternative of a non-zero difference between them. Unless otherwise specified, efficacy parameters will be analyzed using a mixed model with repeated measurements (MMRM) to account for the longitudinal nature of the data. These analyses will include the fixed categorical effect of treatment (PIPE-505 or Placebo) and fixed continuous effect of the baseline value of the efficacy variable. Visit will be treated as the repeated measure, and treatment-by-visit and treatment-by-baseline value interaction terms will be

included. Significance tests will be based on least-squares means using a two-sided $\alpha = 0.05$. The primary treatment comparison will be the contrast between treatments at the Month 3 visit.

Supplementary analyses of efficacy data available in the Per-Protocol Set (PPS) will be conducted using the method described above.

9.10 Interim Analysis

No interim analysis is planned in this study.

9.11 Pharmacokinetic Analyses

PK parameters of PIPE-505 will be derived using non-compartmental analysis methods from the concentration-time data for all evaluable subjects. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. Table 6 describes the PK parameters that will be determined from concentrations of PIPE-505 in plasma.

Table 6: Plasma PK Parameters for Analysis

Parameter	Definition
AUC_∞	Area under the plasma concentration-time curve from time 0 to infinity, calculated as $AUC_\infty = AUC_t + C_{last}/\lambda_z$, where C_{last} is the last quantifiable concentration.
AUC_{24}	Area under the plasma concentration-time curve from time 0 to 24 hours, calculated using the linear trapezoidal rule.
C_{max}	Maximum observed plasma concentration.
CL/F	Apparent clearance after extravascular administration, calculated as Dose/AUC_∞ after a single dose.
λ_z	Terminal elimination rate constant, calculated as the negative of the slope of the log-linear regression of the natural logarithm concentration-time curve during the terminal phase.
$t_{1/2z}$	Terminal elimination half-life, calculated as $\ln(2)/\lambda_z$.
t_{max}	Time to reach C_{max}.
V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration, calculated as $(CL/F)/\lambda_z$.

[redacted]

9.12 Sample Size Calculation and Power Considerations

The planned sample size of 24 subjects (18 Active and 6 Placebo) is not based on statistical considerations but is expected to be sufficient to categorize initial safety, tolerability, and PK characteristics of PIPE-505.

[redacted]

Table 7: [redacted]

[redacted]	[redacted]									
[redacted]	[redacted]									
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[redacted]	[redacted]									

[redacted]

[redacted]

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable US regulations, International Conference on Harmonisation Guidelines (ICH) GCP Guidelines, and local ethical and legal requirements.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study Sponsor and designees will undertake their assigned roles for this study in compliance with all applicable industry regulations and ICH Good GCP Guideline E6(R2) (2018).

Visits to sites are conducted by representatives of the study Sponsor or designee to inspect study data, subjects' medical records, and CRFs in accordance with current GCP and the respective local and inter/national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The Sponsor ensures that Local Regulatory Authority requirements are met before the start of the study. The Sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any Regulatory Authority approvals required prior to release of IP for shipment to the site.

10.1.2 Public Posting of Study Information

The Sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating Investigators' names and contact information.

10.1.3 Study Suspension, Termination, and Completion

The Sponsor may suspend or terminate the study or part of the study at any time for any reason. If the study is suspended or terminated, the Sponsor will ensure that applicable regulatory agencies and IRBs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The Investigator must undertake to perform the study in accordance with ICH GCP Guideline E6(R2) (2018), and applicable regulatory requirements and guidelines.

It is the Investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The Investigator

should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The Investigator will maintain a list of appropriately qualified persons to whom the Investigator has delegated significant study-related tasks. *Curriculum vitae* for Investigators and sub-investigators are provided to the study Sponsor (or designee) before starting the study.

10.2.2 Protocol Adherence and Investigator Agreement

The Investigator and any co-investigators must adhere to the protocol as detailed in this document. The Investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an Investigator Agreement to confirm acceptance and willingness to comply with the study protocol.

If the Investigator suspends or terminates the study at their site, the Investigator will promptly inform the Sponsor and the IRB and provide them with a detailed written explanation. The Investigator will also return all IP, containers, and other study materials to the Sponsor. Upon study completion, the Investigator will provide the Sponsor, IRB, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs, to ensure accurate and timely information is provided at all phases during the study, may be done by the Sponsor or designee, Investigator, or for multi-site studies, the Coordinating Investigator according to national provisions and will be documented in the Investigator Agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Case Report Forms

Access to the eCRFs will be provided by the Sponsor or designee and should be handled in accordance with instructions from the CRO.

The Investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation.

All data sent to the Sponsor must be endorsed by the Investigator.

The Clinical Research Associate (CRA)/Study Monitor will verify the contents against the source records. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source documents to be reviewed during this study may include, but are not limited to, questionnaires, laboratory reports, ECG tracings, x-rays, radiologist reports, or reports,

photographs, clinic notes or pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol. Source documents may also include CRFs or electronic devices when information is recorded directly onto such forms or devices.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and exact duplication of the original document.

All key data must be recorded in the subject's medical records.

The Investigator must permit authorized representatives of the Sponsor, the respective national, local, or foreign regulatory authorities, the IRB, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/Study Monitor (and auditors, IRB or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the Sponsor or its representatives, national or local regulatory authorities, or the IRB having access to source data (e.g., subject's medical file, appointment books, original laboratory reports, etc.).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (e.g., the US Food and Drug Administration [FDA], European Medicines Agency [EMA], UK Medicines and Healthcare Products Regulatory Agency [MHRA]) or an auditor).

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the Sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the MHRA, other regulatory authorities, the Sponsor or its representatives, and the IRB/IEC for each site.

10.2.3.4 Financial Disclosure

The Investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the value of the compensation for conducting the study could be influenced by the outcome of the study. The following information is collected: any significant payments from the Sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in IP; any significant equity interest in the Sponsor or subsidiaries as defined in 21 CFR 54 2(b) (2018).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from all study subjects prior to any study related procedures including Screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-ICF authorized representative as applicable is requested to sign the Subject Informed Consent Form or a certified translation, if applicable after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (i.e., a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative as applicable. If applicable, it is provided in a certified translation of the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The Investigator provides the Sponsor with a copy of the consent form which was reviewed by the IRB and which received their favorable opinion/approval. A copy of the IRB written favorable opinion/approval of these documents must be provided to the Sponsor, prior to the start of the study. Additionally, if the IRB requires modification of the sample Subject Information and consent document provided by the Sponsor, the documentation supporting this requirement must be provided to the Sponsor.

10.3.2 Institutional Review Board

It is the responsibility of the Investigator to submit this protocol, the informed consent document (approved by the Sponsor or their designee), relevant supporting information and all types of subject recruitment information to his/her local IRB for review, and all must be approved prior to site initiation.

Prior to implementing changes in the study, the Sponsor and the IRB must approve any revisions of any revised informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the Sponsor (or their delegate) has received written IRB/IEC approval of and copies of revised documents.

The Investigator is responsible for keeping his/her local IRB/IEC appraised of the progress of the study and of any changes made to the protocol, at least once a year. The Investigator must also keep the local IRB informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the Health Insurance Portability and Accountability Act of 1996

(HIPAA). A site that is not a Covered Entity as defined by HIPAA, must provide documentation of this fact to the CRO.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the Sponsor and/or designee reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market PIPE-505; national or local regulatory authorities; and the IRB(s) which gave approval for the study to proceed. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number. However, their initials and date of birth may also be collected and used to assist the Sponsor to verify the accuracy of the data, for example, to confirm that laboratory results have been assigned to the correct subject.

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: To support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Compliance with Law, Audit, and Debarment

By signing this protocol, the Investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of GCP; and all applicable local laws, rules and regulations relating to the conduct of the clinical study.

The Investigator also agrees to allow monitoring, audits, IRB/IEC review and regulatory agency inspection of trial-related documents and procedures and provide for direct access to all study-related source data and documents.

The Investigator shall prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable local laws, rules and regulations; and, for each subject participating in the study, provide all data, and upon completion or termination of the clinical study submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Study documentation will be promptly and fully disclosed to the Sponsor by the Investigator upon request and also shall be made available at the Investigator's site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory agencies. The Investigator agrees to promptly take any reasonable steps that are requested by the

Sponsor as a result of an audit to correct deficiencies in the study documentation and case report forms.

International Conference on Harmonization's GCP guidelines recommend that the Investigator inform the subject's primary physician about the subject's participation in the study if the subject has a primary physician and if the subject agrees to the primary physician being informed.

The Investigator will promptly inform the Sponsor of any regulatory agency inspection conducted for this study and provide the final results (i.e., final observations and responses).

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on this study. The Investigator will immediately disclose in writing to the Sponsor if any person who is involved in conducting the study is debarred or if any proceeding for debarment is pending or, to the best of the Investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular study site, the Sponsor will promptly notify the IRB/IEC.

10.6 Quality Control and Quality Assurance

By signing this protocol, the Sponsor agrees to ensure that studies are conducted and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP under quality assurance systems with written standard operating procedures, and all applicable local laws, rules and regulations relating to the conduct of the clinical study.

10.7 Publication Policy

All manuscripts, abstracts, or other modes of presentation arising from the results of the study must be reviewed and approved in writing by the Sponsor, in advance of submission. The review is aimed at protecting the Sponsor's proprietary information existing either at the date of the commencement of the study or generated during the study.

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