Pharmaceutical Interventions for Noise-Induced Hearing Loss–Acute Exposure Treatment (PINIHL-AET)

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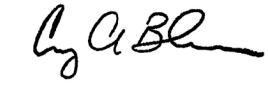
I have read this protocol and agree to conduct this study in accordance with all stipulations of the protocol.

Craig Buchman, MD		03/03/2023
Principal Investigator Name: (Printed)	Signature	Date

Pharmaceutical Interventions for Noise-Induced Hearing Loss–Acute Exposure Treatment (PINIHL-AET)

Protocol Revision History

Version Date	Revision Summary
19 January 2021	Initial Version
19 February 2021	• Clarification of baseline and follow-up blood draws that include an electrolyte panel, BUN, Cr, ALT, and AST; serum pregnancy at baseline • Addition of effective birth control instruction
10 June 2021	• Addition of NIOSH red flag guideline (an increase in hearing threshold level of 15 dB or more at any frequency (2, 3, 4, 6 kHz) in either ear for permanent threshold shift as a secondary endpoint • Addition of LENS-Q Adapted for Surgical Noise Study and stratification language • Surgery drilling time reduced to 45 minutes for inclusion criteria • Change in sample size • Corrected section 5.5 Follow-up Assessments to include "Hearing History and Occupation Exposure" questionnaire as listed in study calendar and appendix E • Study calendar clarifications including baseline testing adjusted from 1 week to 1-2 weeks • Numbering correction in "Hearing History and Occupation Exposure—Visit 1" questionnaire • Edited inclusion criteria for DPOAE as the number of data points (denominator) was incorrect • Removed urine sample; Added blood sample for PGx collection at baseline visit.

	• Range of 2-8 degree Celsius (C) refrigerator at WUSM replaces 4 degree C for blood sample • Removed PI and Sub-Investigator information • List protocol version as a separate line. • Statistical considerations section updated to match SAP
21 October 2021	• Inclusion of stratification table for noise exposure • Randomization language updated
09 November 2021	• Clarification of language for secondary outcomes analysis criteria
21 January 2022	• Change time between baseline visit (#1) and surgical visit (#2) from 1-2 weeks to within 1 month (+/- 3 days).
23 March 2022	• Clarification of post-op DPOAE timing from within 4-8 hours to within 8 hours. • Clarification of baseline visit timing to within 30 days (+3 days). • Update timing of pre- and post-op dosing. • Correct efficacy analysis timing. • Addition of audiometry language in situations of an absence of a threshold.
04 October 2022	• WIN conducted once rather than 3 times. • ECG removed from study calendar and pre-op data collection as it is not standard of care
18 November 2022	• Update audiometric inclusion criteria
03 March 2023	• Correct air-bone gap inclusion criteria • Minor edits for consistency with previous modifications

TABLE OF CONTENTS

1.0 BACKGROUND AND SIGNIFICANCE 6
1.1 Noise-induced hearing loss (NIHL) 6
1.2 NIHL and its Pathogenesis 6
1.3 Molecular Pathways Underlying NIHL 7
1.4 NIHL and Pharmacogenetics 8
1.5 NIHL and Drilling Noise During Skull-based Surgery 10
2.0 STUDY OBJECTIVES 10
2.1 Primary Objective 10
2.2 Secondary Objectives 11
3.0 ELIGIBILITY CRITERIA 11
3.1 Inclusion Criteria 11
3.2 Exclusion Criteria 11
3.3 Inclusion of Women and Minorities 12
4.0 STUDY DESIGN 12
5.0 SCHEDULED ASSESSMENTS 13
5.1 Screening/Baseline/Preoperative Assessment 13
5.2 Randomization 15
5.3 Standard of Care Surgery 17
5.4 Intra-operative Testing 17
5.5 Follow-Up Assessments 17
5.6 Blood collection, transportation, and storage 18
6.0 EVALUABILITY 18
7.0 PHARMACEUTICAL INFORMATION 19
7.1 Zonisamide (ZONEGRAN®) 19
7.2 Placebo 20
8.0 REGULATORY AND REPORTING REQUIREMENTS 20
8.1 Sponsor-Investigator Reporting Requirements 21
8.2 Secondary Site Reporting Requirements 22
8.3 Exceptions to Expedited Reporting 23
9.0 STUDY CALENDAR 24
10.0 DATA SUBMISSION SCHEDULE 24
11.0 DATA AND SAFETY MONITORING 25
11.1 Adverse Event Collection in the Case Report Forms 26
12.0 STATISTICAL CONSIDERATIONS 26
12.1 Data Analysis 26
12.2 Efficacy analysis 26
12.3 Sample Size Estimation 30
12.4 Interim Analysis 31
12.5 Pharmacogenetics analysis plan 32
13.0 REFERENCES 33
APPENDIX A: Definitions for Adverse Event Reporting 44
APPENDIX B: Reporting Timelines 46
APPENDIX C: Washington University Unanticipated Problem Reporting Cover Sheet 48
APPENDIX D: Questionnaire 1a: Hearing History and Occupation Exposure – Visit 1 49
APPENDIX E: Questionnaire 1b: Hearing History and Occupation Exposure – Visit 3 51
APPENDIX F: LENS-Q Adapted for Surgical Noise Study –Visit 1 53

1.0 BACKGROUND AND SIGNIFICANCE

1.1 Noise-induced hearing loss (NIHL)

Noise-induced hearing loss (NIHL) is a serious problem. When a service member leaves the military, hearing loss can impact his or her quality of life and employability (Pfannenstiel, 2014). Service members are vulnerable to two types of NIHL: occupational NIHL due to continuous or intermittent noise exposure and acoustic trauma due to a sudden burst of sound. Because no form of hearing protection offers complete protection against noise of that intensity, repeated firing of weapons even with ear protection devices can subsequently lead to occupational NIHL (Chen and Brueck, 2011). Almost every member of the armed forces will be exposed to hazardous noise at some point in his or her career (McIlwain et al., 2008; Kirchner et al., 2012; Yankaskas, 2013), highlighting the urgent need for pharmaceutical intervention. Despite positive outcomes in preclinical studies, to date, no drugs have been approved by the U.S. Food and Drug Administration (FDA) for use in the amelioration of NIHL (Le Prell and Bao, 2012; Mukherjea et al., 2015).

1.2 NIHL and its Pathogenesis

After noise exposure, two phases of hearing loss can be measured. The first is a temporary threshold shift (TTS), which is greatest immediately after noise exposure, and gradually lessens within the first 24 hours. The second phase is a permanent threshold shift (PTS), which is measured two to three weeks after noise exposure (for recent review, Ryan et al., 2016; Liberman, 2016). These changes are typically monitored using behavioral pure-tone thresholds, distortion product otoacoustic emissions (DPOAE), or the auditory brainstem response (ABR) to generate an audiogram (a plot of threshold as a function of test frequency). The noise-induced damage is dependent on the noise pattern, intensity, and duration, with longer and louder noises being more hazardous than shorter or quieter sound exposures (Wang et al., 2002; Harding and Bohne, 2007; Chen et al., 2015). In addition, NIHL susceptibility differs markedly among individuals, resulting from the interaction of genetic and environmental factors (Clifford et al., 2016; Groth et al., 2016; Lavinsky et al., 2016). For example, in animals, the C57BL/6J mouse strain is more susceptible to noise than other mouse strains (Davis et al., 2001). In humans, individuals with specific single nucleotide polymorphisms (SNPs) in genes for certain antioxidant enzymes may be more susceptible to NIHL (Lin et al., 2009).

NIHL is caused by sensorineural damage, primarily to the sensory hair cells and primary auditory neurons of the cochlea (Liberman, 2017). Outer hair cells (OHCs) are particularly sensitive to noise. When OHCs are damaged, hearing thresholds increase due to a loss in amplification of the cochlear signal. Recently, Kujawa and Liberman (2006, 2009) have expanded on these classic findings with the observation that certain noise exposures at “benign” levels to rodents can result in

only TTS, but no PTS. Nevertheless, the animals show selective synaptic loss between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) with high thresholds, ultimately accelerating hearing loss over time. Because this cochlear synaptopathy does not change the hearing threshold immediately, the term “hidden hearing loss” has been used to label the hidden synaptopathic injury, and this term has also been used to describe corresponding functional deficits that are assumed to be hidden behind the normal hearing threshold.

Clinically, difficulties with understanding speech in noise have long been observed in older adults with normal audiometric thresholds (e.g. Frisina and Frisina, 1997). Loss of fidelity in the encoding of suprathreshold signals may provide one explanation for this deficit (Sergeyenko et al., 2013; Tremblay et al., 2015). Thus, there is the potential for profound functional consequences after a so-called benign noise exposure that led to only TTS. Of particular concern for military personnel with the potential for repeat exposure (Davis, 2016; Bramhall et al., 2017), further studies have found that benign noise exposures resulting in only TTS can also contribute to PTS after repeated exposure (Wang and Ren, 2012), underpinning the importance of developing pharmaceutical interventions to prevent noise-induced cochlear synaptopathy for military service members.

1.3 Molecular Pathways Underlying NIHL

Although mechanical destruction and decreased blood flow contribute to NIHL (Quirk et al., 1991; Mulroy et al., 1998), several key molecular mechanisms such as signaling mediated by an ATP receptor have been identified to contribute to TTS (for recent review, see Kurabi et al., 2017). Common mechanisms underlying both TTS and PTS have also been identified. One is the increase of mitochondrial free radical formation such as reactive oxygen species (ROS) due to noise-induced intense metabolic activity in the cochlea (e.g., Yamane et al., 1995; Ohlemiller et al., 1999; Ohinata et al., 2000; Henderson et al., 2006; Campbell et al., 2007; Darrat et al., 2007). Thus, it is not surprising that attempts to prevent NIHL with antioxidant agents have become the focus of much research in this field (Seidman et al., 1993; Hight et al., 2003; McFadden et al., 2005; Yamashita et al., 2005; for review, see Le Prell and Lobarinas, 2015). However, most of these interventions have been only partially effective or ineffective in preventing NIHL (Lynch and Kil, 2005; Campbell et al., 2007; Kopke et al., 2007; Le Prell et al., 2007). The largely disappointing outcomes may be due to a narrow therapeutic window. As ROS signaling is also important for normal cellular function (for recent review, Sbodio et al., 2018), high doses of antioxidants may have less therapeutic benefit (for example, Kil et al., 2017). Recently, new signaling pathways underlying NIHL have been identified, including deregulation of calcium homeostasis (Guitton et al., 2004; Zine and Van De Water, 2004; Chen et al., 2012; Han et al., 2015; Chen et al., 2015). Deregulation of calcium signaling may contribute to development of both TTS and PTS. In addition, calcium signaling is upstream of many other cellular survival signaling pathways. For example, it can control ROS signaling by regulating the release of ROS from the mitochondrion (Estergerg et al., 2013,

2014). Calcium homeostasis in the cochlea can be regulated by several types of calcium channels, which include voltage-gated calcium channels (VGCCs) (Rodrigues Contreraz and Yamoah, 2001; Adamson et al., 2002; Fuchs, 2002; Schnee and Ricci, 2003). VGCCs can be divided into two groups: high-voltage-activated calcium channels and low-voltage-activated calcium channels (Igelmund et al., 1996; Lacinova et al., 2000; Perez-Reyes, 2003; Yunker and McEnery, 2003). The family of low-voltage-activated, or T-type, calcium channels (Cav3) is composed of three members (Cav3.1, Cav3.2, and Cav3.3) based on their respective main pore-forming alpha subunits: α 1G, α 1H, and α1I (Perez-Reyes, 2003; Yunker and McEnery, 2003). Our studies on drug repurposing have shown that a family of antiepileptic drugs blocking T-type calcium channels can prevent and treat NIHL (Shen et al., 2007; Bao et al., 2013). We have also determined the expression pattern of these calcium channels in the cochlea. All subtypes are present in SGNs, and α 1G and α 1I are expressed in the hair cells and supporting cells (Shen et al., 2007). Thus, it is not surprising that an antiepileptic drug (AED), zonisamide (ZNS), which blocks T-type calcium channels, has both prophylactic and therapeutic functions against NIHL (Bao et al., 2013). In addition, epidemiological studies show that ZNS is well-tolerated even for long-term treatment (Hashimoto et al., 1994; Leppik, 2006, White et al., 2010). These findings have led us to this project, which is the repurposing ZNS against NIHL for military service members.

1.4 NIHL and Pharmacogenetics

Preliminary results. Pharmacogenetics (PGx) is the study of how a person's genetic makeup determines his or her response to a therapeutic intervention. It offers the promise of utilizing genetic fingerprints to predict an individual's responses to drugs in terms of safety, efficacy, and pharmacokinetics. It can revolutionize the practice of medicine by individualizing treatment through the use of novel diagnostic tools. To date over 100 loci have been associated with syndromic and non-syndromic hearing loss providing excellent biomarkers for PGx studies. These markers are easily surveyed through both SNP-based or whole exome sequencing using DNA samples taken from patients (Pawelczyk et al. 2009; Konings et al. 2009; Grondin et al. 2015). In a recent study, a genetic risk score for the likelihood of NIHL was developed based on SNP markers in 10 genes (Zhang et al. 2019). Thus, we will test the applicability of this index to predicting both TTS and PTS in patients undergoing skull-base surgery. In addition to examining associations of candidate genes for NIHL, we will also screen patients for known genetic variation associated with the metabolism of ZNS (Saruwatari et al. 2010). Ultimately, we envision incorporating SNPs associated with high risk for NIHL with drug metabolism SNPs to develop predictive models for drug efficacy. These data would be used in dialogue with FDA and subsequent studies to develop personalized drug treatment regimens for patients.

Here, we provide three types of data from our PGx study of age-related hearing loss (ARHL). They are highly pertinent because the same approaches will be applied to this project. First, we describe our recent clinical findings on the delay of ARHL in human subjects taking calcium channel blockers (CCBs). Second, we present our preliminary human genetic studies of ARHL in the same population based on the continuous extreme phenotypes (CEP) and sequence kernel association test (SKAT) approaches. Third, we present an estimate of patient populations using the CEP-SKAT method.

Delay of ARHL in patients taking CCBs. In our preliminary study, a total of 35 white female patients have completed their first visit, with 26 of them using amlodipine (74%) for more than one year. We compared this CCB group with two control cohorts, also of white females: control 1 group (Con 1) from the Rochester, NY, area (447 participants) and control 2 group (Con 2) from the St. Louis, MO, area (55 participants) (**Fig. 1**).

Fig. 1. CCB protection against ARHL. Participants taking CCBs show better hearing thresholds than participants taking no CCBs even at low frequency regions (0.25 to 1 kHz).

Table 1. Least Squares Means with Bonferroni Correction Model 1

Cohort	LS Mean	Vs. Control 1 p-value	Vs. Control 2 p-value
CCB	19.77	1.0000	.9202
Control 1	20.28		.0784
Control 2	17.50		

Since ARHL starts at higher frequencies in the cochlea, we divided audiograms into three pure tone averages (PTA): averages of 0.25, 0.5, and 1 kHz (PTAL); averages of 2 and 4 kHz (PTAH24); and averages of 2, 4, and 8 kHz (PTAH248). The means for the CCB group were: PTAL (15.1 dB HL), PTAH24 (20.7 dB HL), and PTAH248 (24.0 dB HL), and the means for the control 1 group were: PTAL (20.1 dB HL), PTAH24 (30.7 dB HL), and PTAH248 (35.2 dB HL). Since there were no data for 8 kHz for the control 2 group, the means for this group were PTAL (20.7, dB HL) and PTAH24 (29.3 dB HL). The two-tailed unequal variance t-test showed a significant difference between the CCB and the control 1 group for PTAL (p = 0.00067), PTAH24 (p = 0.00001), and PTAH248 (p = 0.00021), and a significant difference between the CCB and control 2 groups for PTAL (p = 0.02777) and PTAH24 (p = 0.00959). To correct for possible influences from both age and the three cohort sites, we used multivariate regression models with the Bonferroni correction method (**Table 1**, **2** and **3** for PTAL, PTAH24 and PTAH248, respectively). No significant difference was observed for PTAL between the CCB and control 1 or 2 groups (**Table 1**), or PTAH24 (**Table 2**) between the CCB and

Table 2. Least Squares means with Bonferroni Correction Model 2

Cohort	LS Mean	Vs. Control 1 p-value	Vs. Control 2 p-value
CCB	28.17	.0420*	1.0000
Control 1	35.19		.11
Control 2	28.54		

Table 3. Least Squares Means with Bonferroni Correction Model 3

Cohort	LS Mean	Vs. Control 1 p-value
CCB	32.41	.0145*
Control 1	39.28	

control 2 group. However, statistically significant differences were found for both PTAH24 and PTAH248 between the CCB and control 1 group (**Table 2** and **3**). These data indicate CCB had beneficial effects against peripheral ARHL.

1.5 NIHL and Drilling Noise During Skull-based Surgery

Patients undergoing skull-based surgery are unavoidably exposed to noise from operative drills. High-speed drills that are capable of producing elevated levels are used during skull based surgeries (Hilmi et al., 2011; Yu et al., 2014). Hilmi et al. (2011) used accelerometers with cadaver temporal bones to measure the bone-transmitted drill noise. The authors found that the while the highest overall bone-conducted noise levels occurred while drilling on the mastoid process with a sound level of 110.2 dBA, other drilling locations such as the cranial base produce excessive amounts of drill noise primarily in the 2-4 Hz bandwidth. If bone-transmitted surgical drilling noise exposure was regulated by occupational safety organizations such as the National Institute of Occupational Safety and Health (NIOSH), the exposure time duration would be limited to between 1.5 to 12 minutes depending on drill location.

The drilling noise transmitted to the unoperated ear through the skull is difficult to prevent. Thus, noise-induced damage cannot be avoided by mechanical protection such as ear plug. Many patients who undergo a skull-based surgery for treatment to an affected ear, have normal or near-normal hearing in the contralateral ear. Therefore, the unoperated, normal-hearing ear is subjected to a similar drill-noise intensity compared to the operated side (Tos et al., 1984; Hickey and O'Connor, 1991). It is not surprising that temporary cochlear changes are commonly observed in unoperated ears. It has been shown that surgical drilling may have a temporary effect on the amplitude of the otoacoustic emissions of the ear contralateral to the surgical site (Baradaranfar et al., 2015; Shenoy et al., 2015). Furthermore, it has been reported that the incidence of a permanent SNHL (PTS) following tympanomastoid surgery is between 1.2% and 4.5% (Tos et al., 1984). PTS is also observed in some studies (Palva and Sorri, 1979; Lustig et al., 1995; Hallmo and Mair, 1996; Goyal et al., 2013; Abtahi et al., 2016).

The overarching goal of this study is to test whether ZNS can prevent temporary cochlear changes and PTS in patients undergoing skull-based surgery. Participants will be randomized to receive either active treatment (ZNS) or placebo.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

To determine if preoperative and/or postoperative ZNS is more effective than placebo at preventing PTS in the contralateral ear of patients undergoing drilling during skull base surgery.

2.2 Secondary Objectives

To determine if preoperative ZNS is more effective than placebo at preventing temporary changes in cochlear health, synaptopathy, and degraded speech perception in the contralateral ear of patients undergoing drilling during skull base surgery.

To identify a genetic risk profile associated with drilling-induced hearing loss we will survey known genetic markers associated with NIHL including markers in CDH23, PCDH15, EYA4, MYO1A, KCNMA1 and OTOG (Zhang et al., (2019) and zonisamide (ZNS) metabolism, CYP2C9 and CYP2C19 (Saruwatari et al., 2010). If significant associations are observed and validated in a subsequent clinical study, we will optimize drug dosages based on a subject's genetic profile.

3.0 ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

1. Patients who are scheduled to undergo a skull-based surgery that requires at least 45 minutes of surgical-drilling.

2. At least 18 years of age.

3. Air conduction thresholds in the non-operated ears are to be no worse than 25 dB HL for pure tone average 0.5, 1, and 2 kHz with no individual threshold greater than 30 dB HL, and no worse than 45 dB HL at 4 kHz at screening.

4. Observed air-bone gap ≤ 10 dB HL at 0.5, 1, 2, and 4 kHz, with normal tympanometry.

5. Ability to understand and willingness to sign an IRB approved written informed consent document.

3.2 Exclusion Criteria

1. History of known sulfa allergy or hypersensitivity to carbonic anhydrase inhibitors.

2. History of moderate-to-severe kidney or liver disease.

3. Acute viral, bacterial, fungal or parasitic infection.

4. History of seizures.

5. Currently pregnant or breast-feeding.

6. Any current or history of ear disorder and/or central auditory dysfunction in the non-operated ear.

7. History of ototoxic drug use.

8. Current use of strong/moderate 3A4 inhibitor/inducer and grapefruit juice.

Note: For secondary outcomes analysis only, exclusion criteria is as follows:

a) DPOAE data will be used as a secondary outcome measure, and participants will be excluded if their DPOAE is absent at more than 4/10 frequencies. Criteria for a present response is any response that is > 5 dB SPL above the noise floor and replicable within ±5 dB SPL.
b) ECochG: Participants will be excluded if the ECochG/ABR wave I response is absent.
c) WIN test: Participants with WIN scores greater than moderate difficulty or 14.9 dB SNR will be excluded.

Participants will not be excluded from the study for not meeting secondary outcome criteria.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 STUDY DESIGN

This study is a randomized, double-blinded placebo-control trial with three parallel groups, to make use of a common control group. Subjects will be randomized in a balanced fashion into one of 3 arms: ZNS 100 mg pre-op, Placebo, or ZNS 100 mg post-op.

The primary efficacy endpoint will be the proportion of PTS-positive subjects defined as the ratio of PTS-positive subjects to total number of subjects within each study arm/group. Subjects defined as PTS-positive will demonstrate an increase in threshold that is ≥10 dB HL at any frequency from 2-6 kHz post-surgery as compared to baseline audiogram.

The secondary efficacy outcome measures will be: (1) the proportion of PTS-positive subjects as defined above, but the definition of PTS will also include the NIOSH red

flag guideline for permanent threshold shift: an increase in hearing threshold level of 15 dB or more at any frequency (2, 3, 4, 6 kHz). (2) The rate of temporary cochlear change as measured by a DPOAE amplitude shift at any frequency that is significantly greater than the stability of each measurement (i.e., 95% confidence interval of each measurement do not overlap). The rate of DPOAE shift is the ratio of DPOAE shift-positive subjects to total subjects within each arm.

To ensure double-blinding of the trial, each subject will be randomized to one of three treatment arms via an interactive randomization tool (IRT) and assigned to study group. Once randomized, each subject will be provided a kit on the day of surgery that contains two bottles, with one package labeled to be taken prior to surgery and another package designated to be taken within 12 hours after surgery or when the patient is released clinically to oral medication. For subjects randomized to "ZNS pre-op", the pre-op package will contain one ZNS capsule (100 mg PO) and the post-op package will contain one placebo capsule that looks, smells, and tastes the same as ZNS capsules. For subjects randomized to "ZNS post-op", the pre-op package will contain one placebo capsule and the post-op package will contain one ZNS capsule (100 mg PO). For the subjects randomized to "placebo", both pre- and post-op packages will contain placebo.

The study will be "masked" or "blinded" in the sense that all the study participants and the study team members will be blinded to the assignment in the study groups. Only the pharmacist who will prepare the study drug kits and the unblinded Statistician will have access to the kit assignments. A copy of the randomization kit list with study ID assignments will be saved in a limited access folder on a secure network server at Pharm-Olam. The Medical Monitor will be contacted in emergent medical cases when knowing the treatments assignment is mandatory for clinical care of the patient.

At the time when we will need to "freeze" the data sets for purposes of developing the DSMB report, a series of SAS programs will be run from an independent programmer to produce data for each pairwise comparison into two subsets of data from the total cohort. Each subset will be de-identified. A previously prepared SAS code will be run in each subset and the output will be used to complete the table shells where the groups will not be identified. The programmer preparing subsets will not be involved in the handling of data forms or the analysis of data.

Study participants will be recruited from the Washington University Otology clinics. Patients will be offered participation if they are being offered skull-based surgery as part of their standard treatment. These are patients that would be recommended skull-based surgery despite this investigation and this investigation will have no influence on treatment recommendations. Consenting and eligible participants will be scheduled for standard of care surgery.

5.0 SCHEDULED ASSESSMENTS

5.1 Screening/Baseline/Preoperative Assessment

The screening and baseline assessments will occur within 30 days (+3 days) of surgery and include the following tests or procedures:

1. Documentation of demographic information, including gender, age, allergies, current medications, imaging related to surgery, and planned surgical approach.

2. Clinical examination of the ears.

3. Documentation of key clinical data such as confirmation of air conduction thresholds in the non-operated ears to be no worse than 25 dB HL for pure tone average 0.5, 1, and 2 kHz with no individual threshold greater than 30 dB HL, and no worse than 45 dB HL at 4 kHz and absence of ear disorder in the ear contralateral to surgery.

4. Collection of laboratory test results that include a pregnancy test*, electrolyte panel, BUN, Cr, ALT, and AST.
   If these tests are not performed as part of pre-operative workup, we will obtain them for research purposes.
   *Women capable of becoming pregnant will be asked to have a pregnancy test before beginning this study. If a pregnancy test (urine or serum) is performed as part of pre-operative testing we will collect the results from the medical record for research purposes. Women capable of becoming pregnant will be instructed to use effective birth control methods and not to become pregnant while participating in this study as there may be unknown risks to the unborn child. There may be long-term effects of the treatment being studied that could increase the risk of harm to an unborn child. The study team must be notified if the birth control method fails while on the study and/or if the participant becomes pregnant while participating in this research study.

5. Blood sample will be collected for PGx surveys.

6. DPOAE, Audiogram, electrocochleography (ECochG), and WIN testing to document the measurements of the non-operated ear.

   Distortion Product Otoacoustic Emissions (DPOAE): DPOAEs are a measure of outer hair cell function and will be used as an indicator of changes in cochlear health and possible PTS in the early period following noise exposure. A soft earphone will be inserted into the participant's ear and a series of tones at a comfortable volume will be played at varying frequencies. No participation is required of the participant as DPOAE are an objective assessment of cochlear health. The measurement system will record the level of the emissions evoked by two primary tones, f1 and f2 (f2/f1 = 1.22) at levels 65 and 55 dB SPL respectively. The f2 primary tone

will be swept from 1-6 kHz, and will be repeated at least five times per session in order to calculate the stability of the emission at each session. All data will be identified and stored on a password protected computer. DPOAE recording will take about 20 minutes to complete.

Audiometry: Audiogram will be performed to look for PTS. Earphones will be placed over the participant's ear and a series of tones at a soft volume will be played at varying frequencies, and the participants have to indicate that they hear the tones by pressing a button. Thresholds will be measured from 250 Hz to 16 kHz. If there is an absence of a threshold at the limits of the equipment, the threshold will be reported as equipment limits (in dB HL) + 10 dB HL. As with the DPOAE, all equipment and procedures are based on a clinically approved protocol.

Electrocochleography (ECochG): An ECochG is an electrophysiological measurement of the cochlea in response to sound and it will be used to identify synaptopathy. It is a clinically-approved auditory evoked potential that is used to evaluate the status of the both the cochlear and the auditory nerve fiber. This measurement is obtained by inserting a soft gold-foiled earphone into the participant's ear. This earphone serves to deliver a series of clicks, as well as an electrode to measure the electrophysiological response of the cochlea to the sound. The electrode montage is completed with a ground electrode on the forehead at midline and a gold-foil electrode in the contralateral to serve as the inverting electrode. The impedance between electrodes will be < 3 kΩ for all participants. The click stimuli at 90 dB nHL used for the study will be repeated 2000 times so that the recording signal can be averaged with artifact rejection. The measurement will be repeated three times. Testing time 45 min to 1 hour.

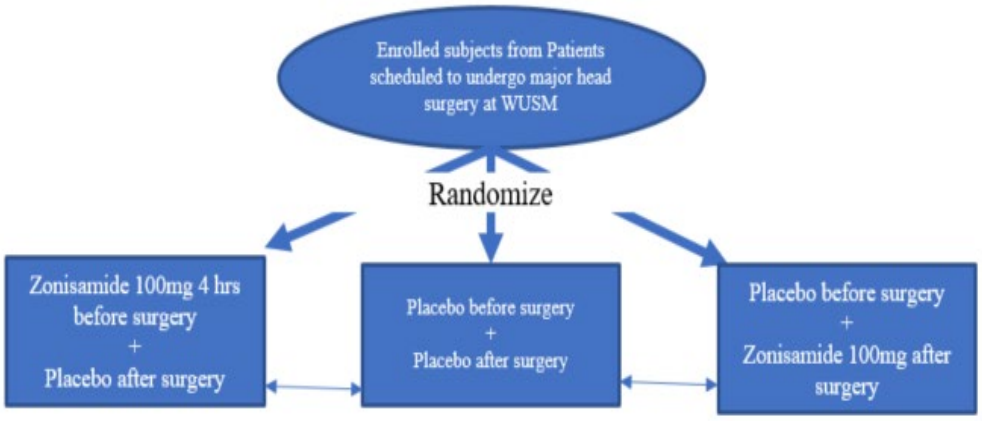
Words in Noise Test (WIN): Earphones will be placed over the participant's test ear and the WIN test will presented to the test ear. The WIN test battery consists of 35 words that are presented with varying degrees of signal-to-noise ratios (SNR) from 24 dB HL to 0 dB HL. The SNR at 24 dB HL is the easiest, with words presented at 24 dB above the babble background, whereas the SNR of 0 dB is the most difficult due to the target words being presented at the same level as the background noise (Wilson and Burks, 2005; Wilson and Watts, 2012). The WIN list will be presented to the test ear one time. The total number of words correctly identified will be used to calculate a dB HL S/N threshold by the Spearman-Karber equation at the mean of 50% correct points. All of speech testing will take 5-10 minutes to complete.

7. Completion of the Hearing History and Occupation Exposure questionnaire and LENS-Q Adapted for Surgical Noise Study.

5.2 Randomization

Participants will be randomized into one of three groups as shown below:

	Pre-op Package	Post-op Package
Pre-op group	ZNS 100 mg PO	Placebo
Placebo group	Placebo	Placebo
Post-op group	Placebo	ZNS 100 mg PO



Randomization will be based on a randomization list generated by unblinded statistician using a computer algorithm written in SAS using randomly selected blocks of sizes 3. Within each block of 3, there will be 1 subject assigned to each study group. To balance noise-exposure history across study arms we will employ stratified randomization. The subjects will be stratified based on noise exposure survey responses (see table below), and will then be randomized to study groups. The random assignment of subjects to the different study groups will be associated with consecutively assigned random numbers which will be unique for each study participant. The stratified kit list will be provided to Advanced Rx who will package and label the drug for shipment to the pharmacist. Each kit will contain two bottles and will be labeled with the same kit number. The bottle will not contain any information of the treatment allocation. One interim analysis is planned, once 33% (n=78) of the subjects have completed participation in the study.

Study medication will be dispensed to the participants with instruction to take their first assigned dose prior to surgery. Participants will take their second assigned dose within 12 hours after surgery or when the patient is released clinically to oral medication. Participants will be instructed to take zonisamide without food. We will recommend that capsules be swallowed whole per the current approved labeling.

Name	**Description**
1: High Noise	Screening: LENS-Q Adapted for Surgical Noise Study –

Name	Description
	1A Or 2A Or 4A = a.Daily, Or b.Less than daily/more than weekly, Or c.Weekly, Or d.Less than weekly/more than monthly Or e.Monthly OR 1C Or 2C Or 3C >= 5 Years
2: Low Noise	Screening: LENS-Q Adapted for Surgical Noise Study – 1A AND 2A AND 4A = f.Less than monthly/more than yearly, g.Yearly, h.Less than yearly Or i.Never OR 1C AND 2C AND 3C < 5 Years

5.3 Standard of Care Surgery

Patients will undergo a typical preoperative workup in preparation for surgery, including routine lab work, pregnancy testing (females only), and a preoperative assessment by anesthesiology. Patients will undergo their procedure per surgical protocol.

5.4 Intra-operative Testing

Documentation of drilling noise, as recorded by a probe mic in the contralateral ear to capture only ambient OR noise intensity level and duration from the non-operated ear during surgery. Documentation will also include duration of anesthesia.

5.5 Follow-Up Assessments

The follow-up assessments will occur after surgery and include the following tests or procedures:

1. DPOAE testing in recovery, within 8 hours after surgery.

2. Blood samples taken within 12 hours following the first pill for PGx surveys.

3. Collection of laboratory test results for research purposes that include an electrolyte panel, BUN, Cr, ALT, and AST. If these tests are not performed as part of clinical care post-operatively, we will obtain them for research purposes at the time of the PGx blood draw.

4. DPOAE, Audiogram, ECochG, and WIN testing 30 days (+/-3 days) after surgery.

5. Completion of the Hearing History and Occupation Exposure questionnaire 30 days (+/- 3 days) after surgery.

6. Documentation of adverse events.

Follow-up assessments will be planned at the stated time points; actual follow up times may vary due to patient logistics and compliance.

5.6 Blood collection, transportation, and storage

Blood samples will be collected from each participant during the baseline visit and the post-surgery visit within 12 hours after the pre-operative drug dose. A post-operative nurse will draw blood into a red-top tube (EDTA or citrate) using an IV line. Samples will be labeled with a study identification number. They will be stored in a 2-8ºC degree refrigerator at WUSM. Samples will then be transported weekly by a study team member for extraction and stored in a -80ºC freezer at Gateway Biotechnology's lab.

6.0 EVALUABILITY

All participants are evaluable for the primary outcome – the proportion of patients who are PTS positive as defined by the ratio of the number of participants with $\geq$ 10 dB increase in PTS to the total number of participants tested 30 days (+/- 3 days) post-surgery – provided they have had the assigned study dose and undergone the post-op study assessments.

Participants who receive the study medication are evaluable for toxicity related to the drug. Participants are evaluated from the time of dose administration through two weeks post dose for drug related adverse events.

The participant will be withdrawn from the study if:

- Participant withdraws consent
- Investigator removes the participant from study
- The Sponsor decides to close the study

7.0 PHARMACEUTICAL INFORMATION

7.1 Zonisamide (ZONEGRAN®)

7.1.1 Zonisamide Description

Molecular formula: $C_8H_8N_2O_3S$
Molecular weight: 212.23

7.1.2 Clinical Pharmacology

The precise mechanism(s) by which ZNS exerts its antiseizure effect is unknown. ZNS may produce these effects through action at sodium and calcium channels. In vitro pharmacological studies suggest that ZNS blocks sodium channels and reduces voltage-dependent, transient inward currents (T-type Ca^{2+} currents), consequently stabilizing neuronal membranes and suppressing neuronal hypersynchronization. Additional information can be found in the package insert.

7.1.3 Supplier

ZNS will be supplied through Advanced Rx (Fort Washington, PA).

7.1.4 Dosage Form and Preparation

ZONEGRAN® is commercially available for oral administration as capsules containing 25 mg, 50 mg, or 100 mg of ZNS.

Each 100 mg capsule contains the labeled amount of ZNS plus the following inactive ingredients: microcrystalline cellulose, hydrogenated vegetable oil, gelatin, and titanium dioxide.

7.1.5 Storage and Stability

Store at 25°C (77°F), excursions permitted to 15–30°C (59–86°F), in a dry place and protected from light.

7.1.6 Administration

For subjects randomized to "ZNS pre-op", the pre-op package will contain ZNS capsules (100 mg) and the post-op package will contain placebo capsules that look and taste the same as ZNS capsules. For subjects randomized to "ZNS post-op", the pre-op package will contain placebo and the post-op package will contain ZNS (100 mg). For the subjects randomized to "placebo", both pre- and post-op packages will contain placebo. Participants will be instructed to take zonisamide without food. We will recommend that capsules be swallowed whole per the current approved labeling.

7.1.7 Side Effects

Potential side effects from the administration of ZNS:

- Somnolence
- Anorexia
- Dizziness
- Ataxia
- Agitation/irritability
- Difficulty with memory and/or concentration

ZNS may cause serious side effects, including:

- Serious skin rash that can cause death.
- Serious allergic reactions that may affect different parts of the body.
- Less sweating and increase in body temperature (fever).
- Suicidal thoughts or actions in some people.
- Increased level of acid in blood (metabolic acidosis).
- Problems with concentration, attention, memory, thinking, speech, or language.
- Blood cell changes such as reduced red and white blood cell counts.

7.2 Placebo

The placebo will contain microcrystalline cellulose which is the predominant filler in the generic capsule.

8.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below. Please refer to Appendix A for definitions and Appendix B for a grid of reporting timelines.

Adverse events will be tracked for the WU site from the time of dose administration through two weeks post dose and at the follow up 30 day visit for drug-related adverse events. All adverse events will be documented and assessed for relatedness to the study medication.

The study team will monitor for adverse events on an ongoing basis. Once the team becomes aware of an adverse event, the AE will be reported according to institutional guidelines. Reporting requirements for Washington University study team may be found in Section 8.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 8.2.

In the event of a Serious Adverse Event determined by the PI to necessitate the breaking of the blind, the intervention assignment will be revealed by the independent programmer to the medical staff doctor caring for the patient. In the event the statistician is unable to be reached in a time needed, to assure the safety of the subject, the blind can be broken by Sara Kukuljan, RN and information will be shared with the medical staff assuming care for the research subject.

8.1 Sponsor-Investigator Reporting Requirements

8.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change

8.1.2 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug

    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

8.1.3 Reporting to Secondary Sites

The Washington University Sponsor-Investigator will notify the research team at the secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the Sponsor-Investigator of the event. This includes events that take place both at Washington University and at other site, if applicable.

8.2 Secondary Site Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator of all serious adverse events (refer to Appendix A, Section D) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place

via email if there is not yet enough information for a formal written report (using FDA Form 3500a (MedWatch) and Washington University's cover sheet (Appendix C). A formal written report must be sent to the Washington University Sponsor-Investigator and designee within **4 calendar days** (for fatal or life-threatening suspected adverse reactions) or **11 calendar days** (for serious unexpected suspected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at the secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

8.3 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 1.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

9.0 STUDY CALENDAR

	Screening / Baseline	Surgery	Post-op	
	Within 30 days (+3 days) prior to surgery		**Within 12 Hours**	**30 days (+/- 3 days)**
Informed consent	X			
Clinical Exam	X			
Demographic Info	X			
Pre-Op Data (pregnancy test, lab results (electrolyte panel, BUN, Cr, ALT, and AST))	X			
Current Meds	X			
Questionnaire	X			X
Audiogram	X			X
ECochG	X			X
DPOAE	X		X*	X
WIN	X			X
Randomization	X			
Oral Dose**		X	X	
Blood draw***	X		X	
AE assessment		X	X	X

* Within 8 hours

** Dispensed on the day of surgery with instruction to take first dose prior to surgery and second dose after surgery.

*** Plasma sample for PGx will be collected at baseline visit. Plasma sample for ZNS level, PGx, electrolyte panel, BUN, Cr, ALT, and AST will be collected within 12 hours after first dose.

10.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to study activities
Eligibility Form	At time of consent; Prior to surgery
Screening/Baseline	Prior to surgery
Surgery/Post-Op Form	Post-Op (within 12 hours)
Post-Op	Post-Op (30 days (+/- 3 days)
Adverse Event Form	Continuous

11.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the PI. The DSM report will be prepared for the DSMB semi-annually and at other times at the discretion of the DSMB. The DSMB must meet at least every six months beginning six months after enrollment of the first participant at the secondary site, no more than one month prior to the due date of the DSM report to the PI. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site, and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Power analysis and/or interim analysis (if described in the protocol)
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO according to institutional guidelines (please refer to Section 1.0).

11.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment must be captured in the AE Form.

12.0 STATISTICAL CONSIDERATIONS

12.1 Data Analysis

Data will be analyzed using an intention-to-treat principle with patients analyzed in the groups they were randomized to. The primary outcome measure for assessing effectiveness of ZNS (100 mg PO) pre-op will be the proportion of subjects in the pre-op arm experiencing PTS as compared to the control group. The primary outcome measure for assessing effectiveness of post-op ZNS (100 mg PO) will be the proportion of subjects in the "post-op" arm experiencing PTS as compared to the control group. The secondary outcome measures are key audiological and clinical assessments of hearing loss. OAE shift will be a secondary outcome measure and an early indicator of changes in cochlear health and PTS.

Standard descriptive statistics will be used to describe distribution of demographic, clinical and audiometric characteristics as well as outcome measures for each study group. For continuous level characteristics Q-Q plots and Shapiro-Wilk test will be used to test assumption of normality. For normally distributed data, mean and standard deviation will be used as descriptive stats of continuous level variables, and if the assumption of normality is violated we will report median and range for description of variables. Frequency and relative frequency will be used for description of categorical level variables.

12.2 Efficacy analysis

12.2.1 Analysis of primary outcome variable

Efficacy analysis at the end of the study

The primary outcome measure for assessing effectiveness of ZNS (100 mg PO) either pre-op or post-op compared to the placebo group will be the proportion of subjects defined as PTS positive 30 days (+/-3 days) after surgery.

Audiogram will be performed to look for PTS. Patients for whom the difference at any frequency from 2-6 kHz in hearing thresholds (30 days (+/-3 days) post-surgery - Baseline) is ≥10 dB HL will be defined as PTS

positive. Primary analysis and sensitivity analyses will be carried out on the primary endpoint.

Primary analysis.

Frequency and relative frequency will be used to describe the distribution of the primary outcome measure in each study group. To assess efficacy, Fisher's exact test will be used to compare the proportion of subjects with PTS positive in ZNS group with the proportion of patients with PTS positive in the placebo group.

To provide an estimate of treatment effect a supportive logistic regression analysis will be done with covariates: age, pre-op hearing dichotomized to normal to minimal hearing loss and slight to mild loss, noise exposure history dichotomized to high risk and low risk, exposure categorized as low (8-hour equivalent A-weighted sound level in decibel (LAeq8hr) < 80 dBA), moderate (LAeq8hr > 80 dBA but < 90 dBA), or high (LAeq8hr > 90 dBA).

Sensitivity analysis of the primary endpoint

The potential impact of missing primary endpoint data will be explored in sensitivity analyses using multiple imputation. Two sensitivity analyses performed:

Sensitivity 1: MI analysis under the missing not at random assumption (MNAR)
Sensitivity 2: Logistic regression tipping point analysis under the assumption of data missing at random.

Sensitivity 1.
Proc MI procedure in SAS will be used to impute missing data for study treatment groups using the distribution implied by the non-missing patient data within the placebo group. The SAS code to impute data for Sensitivity analysis 1 under the MNAR assumption will be of the form:

```
PROC MI DATA=X SEED=<value> NIMPUTE=10  OUT=MI_OUT1 NOPRINT;
CLASS  GROUP;
VAR AGE PTA LSurg……;
FCS LOGISTIC(PTS);
RUN;
```

Post imputation each of the imputed 10 datasets will be analyzed using the same approach as for the primary outcome measure. The estimates of the analysis of the 10 imputed datasets will be then combined following Rubin's rules using PROC MIANALYZE procedure in SAS which will be of the form:

```
PROC MIANALYZE PARMS=GMPARMS COVB=GMCOVB PARMINFO=GMPINFO WCOV
BCOV
    TCOV; / *dataset "gmparms" contains the estimates and
associated standard errors for the mean parameters from each of
the M=10 imputed data sets.
dataset "gmcovb" contains the asymptotic covariance matrics
dataset "gmpinfo" contains parameter info*/

    MODELEFFECTS INTERCEPT AGE PTA LSurg……;
RUN;
```

Sensitivity 2.

Multiple imputation will be used to impute data in each of the study groups. A progressive penalty of $\delta_i = k_i \times \log(OR)$ will be added to imputed values in ZNS arm where (i) OR is the Odds ratio estimate for ZNS as compared to Placebo from the primary logistic regression analysis and (ii) $k_i = 1, 0.95, 0.90, \ldots 0.05, 0, 1.05, 1.10, \ldots$ thus k ranges from 1 (equivalent to MI approach based on MAR) to 0 (or higher), until the conclusion of the primary analysis is overturned (i.e., p<0.05 is lost at, this value of k_i being the 'tipping point'). Rubin's method will be used to combine the primary endpoint treatment effects across imputations for each value, k_i, of the penalty. Forest plots will be used to graphically display the penalty value that results in loss of statistical significance.

SAS code sample for Sensitivity analysis is provided below:

```
**Step 1: Generate 10  datasets by imputing the missing data**;

PROC MI DATA=X SEED=<value> NIMPUTE=10  OUT=MI_OUT1 NOPRINT;
CLASS  GROUP;
VAR AGE PTA LSurg……;
FCS LOGISTIC(PTS);
RUN;

**Step 2: Generate 10 complete datasets from the 10 monotonized
datasetsin Step 1 for missing values in the drug arm,
subtract DELTA derived above from their imputed data.**;
proc mi data=YYY NIMPUTE=1 SEED=<value> OUT=YYY_shift;
by group;
class group;
var AGE PTA LSurg……;
monotone method=logistic;
mnar adjust(PTS / shift=DELTA adjustobs=(group='1'));
run;

**Step 3: Apply the primary MMRM to the 10 complete datasets in S
tep 2**;
proc genmod data=YYY_shift descending;
by _imputation_;
class group;
model PTS = group AGE PTA LSurg……;
ods output GEEModPEst=gmparms;
run;
```

```
**Step 4: Obtain the pooled inference from 10 sets of estimates
from Step 3**;

PROC MIANALYZE PARMS=GMPARMS COVB=GMCOVB PARMINFO=GMPINFO WCOV
BCOV
    TCOV;
    MODELEFFECTS group;
RUN;
```

12.2.2 Analysis for secondary outcome measures.

The focus of the study is to determine efficacy of ZNS for treatment of acute hearing loss based on the testing of hypothesis for primary outcome. In addition, we will also conduct analysis to evaluate other important audiologic measures. The secondary outcome measures are key audiological and clinical assessments of change in cochlear function and hearing loss: DPOAEs, ECochG, WIN testing. They will be measured as continuous level variables. We do not plan any adjustment of alpha error for multiple comparisons.

Analysis of variance (ANCOVA) will be used for comparison of outcome measures between each of the ZNS groups and placebo study group after controlling for baseline value and age, age, pre-op hearing dichotomized to normal to minimal hearing loss and slight to mild loss, noise exposure history dichotomized to high risk and low risk, exposure categorized as low (LAeq8hr < 80 dBA), moderate (LAeq8hr > 80 dBA but < 90 dBA), or high (LAeq8hr > 90 dBA).

Statistical analyses will be conducted using the SAS software (SAS Institute Inc., Cary, N.C., USA).

Missing Data

Every attempt will be made to ensure data completeness. We do not anticipate much loss to follow-up because of the relatively short time follow-up interval. Conservatively, we would estimate that fewer than 5% of subjects will drop out/withdraw from the study. The participant will be withdrawn from the study follow-up procedures if the participant withdraws consent, or the sponsor decides to close the study.

If any, the loss of data would almost certainly be due to the fact that the subjects refused to complete or did not show up for the assessment of PTS 30 days (+/- 3 days) after the surgery. If the participants reschedules the post-surgery appointment for a later date for any reason, and if this delay is within 30 days of the scheduled date, the data will be considered valid and used in the efficacy analysis. Any measure outside this time window of +30 days will be defined and considered missing data.

Missing PTS at 30 days will be imputed using SAS PROC MI procedure within each treatment group using the distribution implied by the non-missing data for the specific treatment group.

The SAS code below using MICE via the Fully Conditional Specification (FCS) statement will be used to impute the missing data under the missing at random (MAR) assumption.

```
PROC MI DATA=X SEED=<value> NIMPUTE=10  OUT=MI_OUT1 NOPRINT;
CLASS  GROUP;
VAR AGE PTA LSurg……;
FCS LOGISTIC(PTS);
RUN;
```

The estimates of the analysis of the 10 imputed datasets will be then combined following Rubin's rules using PROC MIANALYZE procedure in SAS which will be of the form:

```
PROC MIANALYZE PARMS=GMPARMS COVB=GMCOVB PARMINFO=GMPINFO WCOV BCOV
    TCOV; / *dataset "gmparms" contains the estimates and associated
standard errors for the mean parameters from each of the M=10 imputed
data sets.
dataset "gmcovb" contains the asymptotic covariance matrics
dataset "gmpinfo" contains parameter info*/

    MODELEFFECTS group;
RUN;
```

12.3 Sample Size Estimation

Sample size estimation for primary outcome measure.

The primary efficacy outcome will be the proportion of PTS positive subjects defined as the ratio of PTS-positive subjects to total number of subjects in each study arm/group. Using pilot data from a retrospective chart review of 75 similar patients undergoing ≥1-hour drill noise exposure, we estimate the proportion of PTS positive subjects in the placebo group will be 50%. We hypothesize that the expected effect of ZNS either pre-op or post-op will be a 50% reduction of the proportion of subjects with PTS positive as compared to placebo group. This is the desired clinically import effect for treating hearing loss for single dose of 100 mg ZNS.

The sample size for this study was calculated for the planned comparisons of each of the ZNS groups with the placebo group using a balanced design and a one-sided alpha level of 0.0125.

Based on the data from our retrospective study, using Fisher's exact test, we estimated that 78 subjects in the ZNS group and 78 subjects in the placebo group will be needed to provide us with 80.4 % power to detect a ***50% reduction in the proportion of PTS positive subjects*** *(from 50% to 25%* corresponding to an

absolute proportion difference of 25%) at the 1-sided alpha level of 0.0125 for each ZNS-Placebo group comparison. Our one-sided hypothesis is supported by animal studies, and lack of any evidence of hearing loss as a side effect of ZNS in human studies. A total of 234 subjects will be enrolled to be randomized in this study. We will make all the needed effort to minimize and eliminate drop outs, and due to short term-follow-up of the study subjects in a period where they are under medical care, we do not expect any drop-outs or lost to follow-up.

All sample size calculations were carried out using PROC POWER procedure in SAS 9.4.

12.4 Interim Analysis

A sponsor blinded interim analysis focused on the primary endpoint after 33% of the patients have completed participation in the study (26 in each group). The independent programmer will prepare the datasets for each pairwise comparison (subsets of data) using a pre-prepared SAS code and will freeze them for the interim analysis. To ensure the double blinding of the study the subjects will not be presented in the assigned groups. The blinded statistician will estimate the overall proportion of PTS positive subjects in each group.

For each pair-wise comparison, with 33% information, the trial would be stopped for futility if the interim z-value <= 0.6850 (p=0.49 2-sided) for either or both comparisons, corresponding to a conditional power of ≤10% for each comparison. This design would provide 79.6% overall power (i.e. the probability of passing futility and reaching p<0.0125 for one or both comparisons in the final analysis would be 79.6%).

Based on interim analysis the following actions may be taken:

- Stop only one of the ZNS groups for futility
- Stop the trial (both ZNS groups) for futility
- Continue the trial as planned.

Early stopping rules related to serious adverse events: In the event of a serious adverse event, DSMB will evaluate the association of the serious adverse events with the study arm, break the blind if needed, and if found to be associated with treatment, DSMB will consider the study for revision or stopping.

Serious feasibility or design difficulties: If one year after the start of the trial, <50% of the planned accrual goals are met, DSM and the study team will discuss difficulties in recruitment. Patient remuneration will be revised, if needed. If there are not enough patients meeting inclusion/exclusion criteria, then the criteria will be revised without impacting study objectives. If there are not enough patients that undergo more than 1-hr drilling time during surgery, we will explore

including into the study, patients with drilling time at least 45 minutes, expanding audiometric criteria, or expanding to different institutions.

The overall timing associated with the study is based on our previous clinical study experience. Approximately 50 patients per year receive skull surgery that requires 1 hour or more of drilling time at WUSM. Accounting for participant attrition, we plan to finish the study within 4 years. If participant enrollment numbers are consistently below expectations as determined by the study team and DSMC, plans will be made to add additional sites or adjust study inclusion criteria (e.g. including head/skull surgeries requiring less than 1 hour of drilling time).

12.5 Pharmacogenetics analysis plan

This analysis will be performed at the end of the trial by Gateway Biotechnology. To identify genetic variants with ZNS protection against hearing loss, we will first use univariate logistic regression analyses to identify potential confounding variables: sex, age, Z-scores of drug concentration, Z-scores of noise intensity and duration, and Z-scores of hearing functions measure immediately following surgery. Single-marker allelic association analyses will be conducted on the two imputed data sets in PLINK v1.07. The data will be analyzed with a logistic regression model on the additive continuous dosage of minor alleles from 0 to 2 to account for uncertainty of imputation. We will combine association results in the two cohorts by performing a genome-wide inverse-variance weighting meta-analysis using PLINK v1.07, and assuming a fixed-effect model. Functional annotation of top-associated markers will be performed with R package NCBI2R 1.4.6 (http://CRAN.Rproject. org/package=NCBI2R), and key regional association plots of meta-analyzed results will be generated. To confirm whether these variants are specific to ZNS response, we will apply CEP SKAT to analyze genetic associations based on Z-scores of audiogram average threshold shifts and DPOAE amplitudes. All genetic variants, including both common and rare variants, will be included in this association study. Age, gender, and noise duration and intensity will be adjusted for the analysis. The analysis will also be performed using hearing data collected two weeks following surgery. The only differences will be a) using the Z scores of ECochG AP amplitude, latency, and width as well as WIN score, and b) using average audiogram threshold shifts and DPOAE amplitudes at 30 days post-treatment. We will also include metabolite profiling data to support potential genotypic responses to drug treatments. To control for confounding effects, these models will be adjusted for age, gender, and noise duration and intensity. Finally, the control and ZNS-treated comparisons will be performed using post-hoc comparisons with a Bonferroni adjustment for multiple comparisons of any ZNS versus none. Statistical analyses will be performed using the CEP-SKAT method in R language, and the statistical software SAS version 9.4 for Windows will be used for additional analysis.

13.0 REFERENCES

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APPENDIX A: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death

- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX B: Reporting Timelines

Event	HRPO	FDA
Serious AND unexpected suspected adverse reaction		Report no later than 15 calendar days after it is determined that the information qualifies for reporting
Unexpected fatal or life-threatening suspected adverse reaction		Report no later than 7 calendar days after initial receipt of the information
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.	
Protocol exception	Approval must be obtained prior to implementing the change	
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB		Report no later than 15 calendar days after it is determined that the information qualifies for reporting
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.	
Breach of confidentiality	Within 10 working days.	
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.	

Event	HRPO	FDA
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.
Minor deviation	Report summary information at the time of continuing review.	
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.	
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.	

Event	WU (Coordinating Center)	Local IRB	FDA
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.		
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.		
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations		
Protocol exception	Approval must be obtained prior to implementing the change.		

APPENDIX C: Washington University Unanticipated Problem Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Admission Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**

APPENDIX D: Questionnaire 1a: Hearing History and Occupation Exposure – Visit 1

Hearing History and Occupation Exposure Visit 1

1. In the ear opposite of surgery, do you have any difficulties hearing speech?

□ No □ Yes, sometimes □ Yes, often

2. In the ear opposite of surgery, do you have any difficulties hearing other types of sounds?

□ No Yes, sometimes □ Yes, often

3. Do you have tinnitus, ringing in your ears? □ Yes □ No

If yes,

A. Which ear? □ Right □ Left

B. When did the tinnitus start? _____

1. **Suddenly or gradually? _____**

4. Have you worked in any of the following types of loud-noise occupations?

□ Yes □ No

Circle <u>all</u> that apply, even if very brief:

Logging/lumber industry	Mining	Farming
Factory	Landscape (lawnmower/weedeater/chainsaw)	Printing
Truck Driver	Pilot	Police
Construction	Military	Hunting (gun powder)
Fire Department	Musician/Theatrical performer	

Current Occupation: _____

5. Have you been exposed to loud noise during recreational or leisure-time activities (e.g., gunfire, power tools, boat engines, auto engines, motorcycle, ski-mobile, or loud music)?

□ Yes □ No

If yes, describe: _____

6. Have you undergone any accidental exposure to sudden, intense noise?

□ Yes □ No

If yes,

6a. Please explain: type of noise

_____

6b. Which ear or side of your head was exposed?

_____

6c. Your age at the time of noise exposure _____

7. Do you have any blood relatives who have, or have had, any problems with their *hearing?* □ Yes □ No

If yes, please indicate the nature of their problem(s), and their relationship to you.

_____

8. Do you have any blood relatives who have, or have had, *tinnitus?*

□ Yes □ No

If yes, please indicate the nature of their problem(s), and their relationship to you.

_____

REVIEWED BY

CRC signature _____ Date _____

PI Signature _____ Date _____

APPENDIX E: Questionnaire 1b: Hearing History and Occupation Exposure – Visit 3

Hearing History and Occupation Exposure Visit 3

1. In the ear opposite of surgery, do you have any difficulties hearing speech?

□ No □ Yes, sometimes □ Yes, often

2. In the ear opposite of surgery, do you have any difficulties hearing other types of sounds?

□ No Yes, sometimes □ Yes, often

3. Do you have tinnitus, ringing in your ears? □ Yes □ No

If yes,

C. Which ear? □ Right □ Left

D. When did the tinnitus start? _____

2. Suddenly or gradually? _____

4. Since your surgery, have you been in any of the following types of loud-noise situations?

□ Yes □ No

Circle <u>all</u> that apply, even if very brief:

Logging/lumber industry	Mining	Farming
Factory	Landscape (lawnmower/weedeater/chainsaw)	Printing
Truck Driver	Pilot	Police
Construction	Military	Hunting (gun powder)
Fire Department	Musician/Theatrical performer	Rock Concerts

Woodworking	Listening to music on MP3 players

5. Since your surgery, have you been exposed to loud noise during recreational or leisure-time activities (e.g., gunfire, power tools, boat engines, auto engines, motorcycle, ski-mobile, or loud music)?

□ Yes □ No

If yes, describe: _____

6. Since your surgery, have you undergone any accidental exposure to sudden, intense noise?

□ Yes □ No

If yes, Please explain: type of noise

_____

Which ear or side of your head was exposed?

_____

REVIEWED BY

CRC signature _____ Date _____

PI Signature _____ Date _____

APPENDIX F: LENS-Q Adapted for Surgical Noise Study –Visit 1

LENS-Q Adapted for Surgical Noise Study: The point of this modified survey is to stratify participants into relatively higher and relatively lower exposure groups.

Noise Exposure History Interview Questions

1.

A**. In your occupation**, how often are you exposed to loud noise(s) where you have to shout to be heard? (For example, loud equipment or trucks, loud ship or jet engines, exposure from the rifle range, sirens, K-9 noise, loud crowds, loud music (e.g. concert/band events), loud noise from construction sites)

a. Daily
b. Less than daily/more than weekly
c. Weekly
d. Less than weekly/more than monthly
e. Monthly
f. Less than monthly/more than yearly
g. Yearly
h. Less than yearly
i. Never

B. How likely are you to be wearing hearing protection when this occurs (circle one)?

Never Rarely Sometimes Usually Always

C. How many years have you worked in your occupation? _____

2. Have you served in the military? If yes:

A. How often did your **military service** cause you to be exposed to loud noise(s) where you had to shout to be heard? (For example, loud equipment or trucks, loud ship or jet engines, loud aircraft)

a. Daily
b. Less than daily/more than weekly
c. Weekly
d. Less than weekly/more than monthly
e. Monthly
f. Less than monthly/more than yearly
g. Yearly
h. Less than yearly
i. Never

B. How likely were you to be wearing hearing protection when this occurred (circle one)?

Never Rarely Sometimes Usually Always

C. How many years did you serve in the military? _____

3. A. How often did or do **recreational activities** cause you to be exposed to loud noise(s) where you would have to shout to be heard?

a. Daily
b. Less than daily/more than weekly
c. Weekly
d. Less than weekly/more than monthly
e. Monthly
f. Less than monthly/more than yearly
g. Yearly
h. Less than yearly
i. Never

B. Were you wearing hearing protection when this occurred?

Never Rarely Sometimes Usually Always

C. How many years were you involved with a recreational activity where you had to shout to be heard? _____

4. In either your occupation, your military service, or your recreational activities:

A. How often have you been exposed to sudden intense noise? (For example, shooting range, target practice, hunting, explosions, cannon fire, gun shot, music (e.g. drums), etc.)

a. Daily
b. Less than daily/more than weekly
c. Weekly
d. Less than weekly/more than monthly
e. Monthly
f. Less than monthly/more than yearly
g. Yearly
h. Less than yearly
i. Never

B. Were you wearing hearing protection when this occurred (circle one)?

Never Rarely Sometimes Usually Always

Noise Exposure Group Assignment Based on Interview Questions

- Participants with a response to 1.A., 2.A., or 4.A. of monthly or more frequent exposure are assigned to the higher noise group. Participants with less than monthly exposure are assigned to the lower noise group.
- Participants with a response to 1.C., 2.C, or 3.C. of 5 years or more are assigned to the Higher Noise group.