



# Safety and efficacy of ebselen for the prevention of noise-induced hearing loss: a randomised, double-blind, placebo-controlled, phase 2 trial

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## Summary

**Background** Noise-induced hearing loss is a leading cause of occupational and recreational injury and disease, and a major determinant of age-related hearing loss. No therapeutic agent has been approved for the prevention or treatment of this disorder. In animal models, glutathione peroxidase 1 (GPx1) activity is reduced after acute noise exposure. Ebselen, a novel GPx1 mimic, has been shown to reduce both temporary and permanent noise-induced hearing loss in preclinical studies. We assessed the safety and efficacy of ebselen for the prevention of noise-induced hearing loss in young adults in a phase 2 clinical trial.

**Methods** In this single-centre, randomised, double-blind, placebo-controlled phase 2 trial, healthy adults aged 18–31 years were randomly assigned (1:1:1:1) at the University of Florida (Gainesville, FL, USA) to receive ebselen 200 mg, 400 mg, or 600 mg, or placebo orally twice daily for 4 days, beginning 2 days before a calibrated sound challenge (4 h of pre-recorded music delivered by insert earphones). Randomisation was done with an allocation sequence generated by an independent third party. The primary outcome was mean temporary threshold shift (TTS) at 4 kHz measured 15 min after the calibrated sound challenge by pure tone audiometry; a reduction of 50% in an ebselen dose group compared with the placebo group was judged to be clinically relevant. All participants who received the calibrated sound challenge and at least one dose of study drug were included in the efficacy analysis. All randomly assigned patients were included in the safety analysis. This trial is registered with ClinicalTrials.gov, number NCT01444846.

**Findings** Between Jan 11, 2013, and March 24, 2014, 83 participants were enrolled and randomly assigned to receive ebselen 200 mg (n=22), 400 mg (n=20), or 600 mg (n=21), or placebo (n=20). Two participants in the 200 mg ebselen group were discontinued from the study before the calibrated sound challenge because they no longer met the inclusion criteria; these participants were excluded from the efficacy analysis. Mean TTS at 4 kHz was 1.32 dB (SE 0.91) in the 400 mg ebselen group compared with 4.07 dB (0.90) in the placebo group, representing a significant reduction of 68% (difference –2.75 dB, 95% CI –4.54 to –0.97; p=0.0025). Compared with placebo, TTS at 4 kHz was non-significantly reduced by 21% in the 200 mg ebselen group (3.23 dB [SE 0.91] vs 4.07 dB [0.90] in the placebo group; difference –0.84 dB, 95% CI –2.63 to 0.94; p=0.3542) and by 7% in the 600 mg ebselen group (3.81 dB [0.90] vs 4.07 dB [0.90] in the placebo group; difference –0.27, 95% CI –2.03 to 1.50; p=0.7659). Ebselen treatment was well tolerated across all doses and no significant differences were seen in any haematological, serum chemistry, or radiological assessments between the ebselen groups and the placebo group.

**Interpretation** Treatment with ebselen was safe and effective at a dose of 400 mg twice daily in preventing a noise-induced TTS. These data lend support to a role of GPx1 activity in acute noise-induced hearing loss.

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## Introduction

Noise-induced hearing loss affects workers in many occupations, and can also affect those exposed to loud music and other recreational activities.<sup>1,2</sup> In 2015, WHO estimated that 1.1 billion teenagers and young adults (aged 12–35 years) are at risk for noise-induced hearing loss, as a result of personal music player use at high levels, use of firearms without adequate hearing protection, and exposure to loud sounds in recreational settings such as concerts, clubs, and bars.<sup>3</sup> The biology and epidemiology of noise-induced hearing loss are under extensive investigation, and data from animal models have shown the involvement of several distinct

structures within the cochlea.<sup>4</sup> To date, no investigational or approved drugs have provided a clinically relevant reduction in the incidence or severity of noise-induced hearing loss.

Glutathione peroxidase 1 (GPx1) is highly expressed in hair cells, support cells, spiral ganglia neurons, and the stria vascularis and spiral ligament, and is the dominant GPx isoform in the cochlea.<sup>5</sup> This pattern suggests that GPx1 might be essential for maintaining normal cochlear function in mammals. In support of this hypothesis, deletion of GPx1 in mice increases vulnerability to noise-induced hearing loss<sup>6</sup> and age-related hearing loss<sup>7</sup> relative to wild-type littermates.

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### Research in context

#### Evidence before this study

We searched PubMed multiple times between Sept 30, 2011, and Dec 6, 2012, using the search terms “noise induced hearing loss”, “TTS”, “PTS”, “GPx”, and “ebselen”. No restrictions were applied to language, article type, or publication date. Noise-induced hearing loss is a leading form of sensorineural hearing loss and involves several cellular structures within the inner ear or cochlea. There are no US Food and Drug Administration-approved drugs for the prevention or treatment of noise-induced hearing loss in either the acute or chronic setting. In animal models, glutathione peroxidase 1 (GPx1) is highly expressed in several cell types within the cochlea that are involved in noise-induced hearing loss. After noise exposure, GPx1 expression is reduced in these cochlear structures, resulting in cell injury and death. Treatment with ebselen, a novel GPx1 mimic, reduced the extent of noise-induced cell injury and death in animal models, thereby preventing acute and chronic noise-induced hearing loss.

Different noise exposures (intensity or duration) can result in temporary threshold shifts (TTSs) that occur and resolve over hours to days; these shifts complicate the study of acute noise-induced hearing loss in human clinical studies. TTSs, now recognised as a disorder of the ear in the International Classification of Diseases, revision 10 (ICD-10), can give rise to permanent threshold shifts and chronic noise-induced hearing loss. Additionally, many studies of acute noise-induced hearing loss do not adequately detail the timecourse of the TTS, especially within hours to days of a single noise exposure. The recent development of a clinical model of noise-induced TTS or

acute noise-induced hearing loss indicates that a TTS can be induced with a single 4-h noise exposure (or calibrated sound challenge) that resolves within hours to days. Noise-induced TTSs were identified by comparing baseline hearing thresholds with thresholds after calibrated sound challenge over the course of hours to 2 weeks post noise.

#### Added value of this study

To our knowledge, this is the first controlled clinical trial of an investigational drug to show a reduction in noise-induced TTS compared with placebo in healthy participants. Compared with placebo, treatment with 400 mg ebselen twice daily resulted in a clinically relevant reduction in the severity of noise-induced TTS at 4 kHz as determined by repeat pure tone audiometry 15 min post noise; however, this finding was not seen with the 200 mg or 600 mg twice daily doses. However, clinically relevant reductions were seen with the 200 mg and 600 mg doses at other frequencies. Ebselen treatment was well tolerated across all doses.

#### Implications of all the available evidence

Our finding that acute noise-induced hearing loss was reduced in participants assigned to ebselen compared with placebo lends support to the GPx1 hypothesis. GPx1 might be the first potential otoprotective drug target identified or partly validated in human hearing. In view of the safety, tolerability, and oral route of administration of ebselen, this drug lends itself to future testing in several types of acquired forms of hearing loss including chronic noise-induced hearing loss and perhaps age-related hearing loss.

Human susceptibility to noise-induced hearing loss might also be associated with a single nucleotide polymorphism (SNP) in GPx1.<sup>8</sup> In view of the overlapping role of oxidative stress and cellular injury in noise-induced and age-related hearing loss, GPx1 is also likely to mediate age-related hearing loss.

Noise can elicit either no change in hearing sensitivity or a reduction in hearing sensitivity that can be temporary or permanent, and potentially unilateral or asymmetric. Susceptibility to acute noise-induced hearing loss is dependent on the type of noise exposure, the exposure level and duration, the use of hearing-protective devices, the quiet time or rest interval between repeated exposures, and the severity of hearing loss before the exposure. Recent development of a personal music player exposure or calibrated sound challenge resolves many of these exposure issues,<sup>9–11</sup> including by controlling source, intensity, duration, hearing-protective devices, and head position.

Hearing loss lasting minutes, hours, days, or even weeks after a single noise exposure is deemed a temporary threshold shift (TTS). Hearing loss that does not resolve to baseline hearing level within weeks to months is considered a permanent threshold shift (PTS). Typically, any clinically significant hearing loss measured

30 days after the last noise exposure would be deemed a PTS.<sup>12</sup> Repeat noise-induced TTS might evoke pathological and physiological changes in several crucial cochlear structures and has been linked to a noise-induced PTS.<sup>13</sup> Fluorescence immunolabelling, and light and electron microscopy show changes in both outer and inner hair cells, spiral ganglia neurons, and several lateral wall structures of the cochlea (stria vascularis and spiral ligament) during TTS induction, resolution, and evolution of PTS.<sup>14–16</sup>

The importance of GPx1 activity in reducing or preventing noise-induced hearing loss was established in several independent animal studies of ebselen, a seleno-organic small molecule that mimics GPx1 activity. GPx1 is a catalytic antioxidant enzyme that is highly expressed in auditory hair cells, supporting cells, spiral ganglia neurons, stria vascularis, and spiral ligament, whereas GPx2–4 are expressed at low levels or are absent.<sup>5</sup> Ebselen treatment reduces the severity and duration of a noise-induced TTS and PTS after single and repeat noise exposures.<sup>17,18</sup> Histological analysis shows that ebselen prevents the loss of inner hair cell innervation and afferent dendrite swelling,<sup>19</sup> swelling of the stria vascularis,<sup>5</sup> and the loss of fibrocytes in the spiral ligament,<sup>20</sup> all essential for normal hearing. Additionally,

eb-selen treatment was found to induce GPx1 expression in several cochlear structures immediately after noise exposure that caused both a TTS and PTS.<sup>5</sup> This effect suggests that ebselen is redox sensitive, and affects several auditory structures undergoing active stress or injury in response to noise.

To test whether ebselen provides otoprotection from noise in people, we used a clinically relevant model of noise-induced hearing loss. To induce noise-induced hearing loss, the calibrated sound challenge was presented at approximately 100 decibels A-weighted (dBA) in-ear, a sound level previously shown to induce a TTS that peaks at 4 kHz, 15 min post noise. 4 kHz hearing is highly affected in noise-induced TTS and PTS and is essential for speech discrimination. The objective of this phase 2 study was to determine the safety and efficacy of ebselen at reducing the severity, duration, or incidence of a noise-induced TTS compared with placebo. We also sought to further demonstrate the usefulness of the calibrated sound challenge as a model system of acute noise-induced hearing loss to test potential otoprotective drugs in an efficient and clinically relevant manner.

## Methods

### Study design and participants

This randomised, double-blind, parallel-group, placebo-controlled, phase 2 safety and efficacy trial was done at the University of Florida (Gainesville, FL, USA). The study was done in accordance with International Conference on Harmonization Good Clinical Practice guidelines under an allowed investigational new drug application, and was reviewed and approved by the University of Florida institutional review board, which also acted as the data and safety monitoring board. The institutional review board reviewed the preparation, conduct, and monitoring guidance of the trial. Participants were recruited through print advertising on and around campus. No changes to the eligibility criteria occurred once screening and enrolment began on Jan 24, 2013, and was completed in March 11, 2014. Study participants gave written informed consent.

Eligible participants were healthy adults aged 18–31 years without a history of otological disease and with hearing thresholds that were less than 25 dB hearing level (HL) at all tested frequencies from 0.25 kHz to 8 kHz. Screening of volunteers began with a review of systems and general health via telephone. Family history of noise-induced hearing loss or an individual's history of noise exposure was not determined. If participants passed otoscopic and tympanometric testing then their pure tone audiometric thresholds were assessed for study eligibility.

Conventional pure-tone air-conduction thresholds (0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) were measured with methods and procedures previously described,<sup>10</sup> in which smaller step sizes (2 dB vs 5 dB) were used to determine hearing thresholds at specific frequencies before and after the calibrated sound challenge. Here, the modified Hughson-

Westlake procedure involved descending in steps of 6 dB whenever a response was obtained, and ascending in steps of 2 dB whenever no response was obtained. Bone-conduction hearing thresholds for 0.25, 0.5, 1, 2, and 4 kHz were obtained at screening for all participants with air-conduction thresholds greater than 15 dB HL. If an air-bone gap of more than 10 dB was observed in the same ear at the same frequency, the participant was excluded and ineligible to enrol. One change to the clinical protocol occurred after participant enrolment began. The determination of an air-bone gap was initially started for all screened participants, but then restricted to only those participants with air-conduction thresholds greater than 15 dB. Additionally, if air-conduction thresholds were asymmetric by more than 15 dB at the same frequency (right vs left ear), the participant was excluded and deemed ineligible to enrol.

Other inclusion criteria were vital signs (ie, heart rate, blood pressure, respiration, and temperature) within normal limits at medical examination. Exclusion criteria were exposure to any duration of non-occupational high-level sound (eg, concerts, firearms, fireworks, and power tools) during the 24-h period preceding baseline audiometric testing as revealed in the questionnaire or during the medical examination; participant's report of aural pain, pressure, fullness, or drainage symptoms; pregnancy; other medical or health issues that would preclude voluntary participation in a drug study (exclusion at the discretion of the principal investigator); and previous receipt of any known potentially ototoxic medication.

### Randomisation and masking

Eligible participants were randomly assigned (1:1:1:1) to receive ebselen (SPI-1005) 200 mg, 400 mg, or 600 mg, or placebo twice daily, orally. Randomisation was done with an allocation sequence generated by an independent third party (EMB Statistical Solutions, Overland Park, KS, USA). The allocation sequence was concealed from all participants and personnel involved in the conduct of the study. The clinician who enrolled the study participants was masked to the allocation sequence and the intervention (study drug containing ebselen or matching placebo), as were the clinicians performing the safety and efficacy assessments and the study participants. The study drug was packaged, labelled, and randomly assigned by an independent third party (Catalent Pharma Solutions, Philadelphia, PA, USA) before shipment to the study site. The packaging and appearance of the placebo capsules were identical to those of the active drug. At the study site, the study drug was matched to the independent randomisation schedule and then distributed to each randomised study participant.

### Procedures

Ebselen or placebo was taken twice daily, orally, for 4 days before meals, beginning 2 days before the calibrated sound challenge. The calibrated sound challenge used in

this trial consisted of digital music recordings containing both lyrics and melodies.<sup>9</sup> Participants selected between two different 4-h playlists containing 63 rock songs or 69 pop songs. The songs were loaded on an iPod (Apple, CA, USA), and delivered via ER6i earphones (Etymotic Research, Elk Grove Village, IL, USA). The calibrated sound challenge is a complex real-world noise experience that has been previously documented to induce a TTS in young adults with normal hearing.<sup>10</sup> In an initial study, participants were exposed to one of three noise levels for 4 h with the 100 dBA noise dose inducing a loss of hearing sensitivity of approximately 6·3 dB at 4 kHz, with decreasing loss at 3 kHz and 6 kHz, creating a noise notch.<sup>10</sup> The noise notch was largest at the first post-noise assessment (15 min post noise), and reduced during the next 3 h post exposure, resolving to baseline hearing levels within 24 h. In that study, all participants were reported to have returned to baseline hearing levels by 7 days post noise. The calibrated sound challenge levels were measured directly by coupling the insert earphones to a B&K spectrum analyser (Brüel & Kjær, Nærum, Denmark) as previously described.<sup>10,11</sup> The calibration of the iPod devices and insert earphones was confirmed before study onset, at multiple times during the course of the study, and at the end of the study.

Pure tone audiometry was assessed two times before and six times after the calibrated sound challenge in four clinic visits. At clinic visit 1 (2–7 days before start of treatment) study participants had their first pure tone audiometry assessment to determine hearing thresholds and study eligibility. At clinic visit 2 (2 days after the start of treatment) study participants' baseline thresholds were assessed to compute subsequent threshold shifts, and their first four thresholds after the calibrated sound challenge were then determined (at 15 min, 1·25 h, 2·25 h, and 3·25 h). At clinic visit 3, 24 h or 1 day post-calibrated-sound-challenge thresholds were determined. At clinic visit 4, 1 week post-calibrated-sound-challenge thresholds were determined.

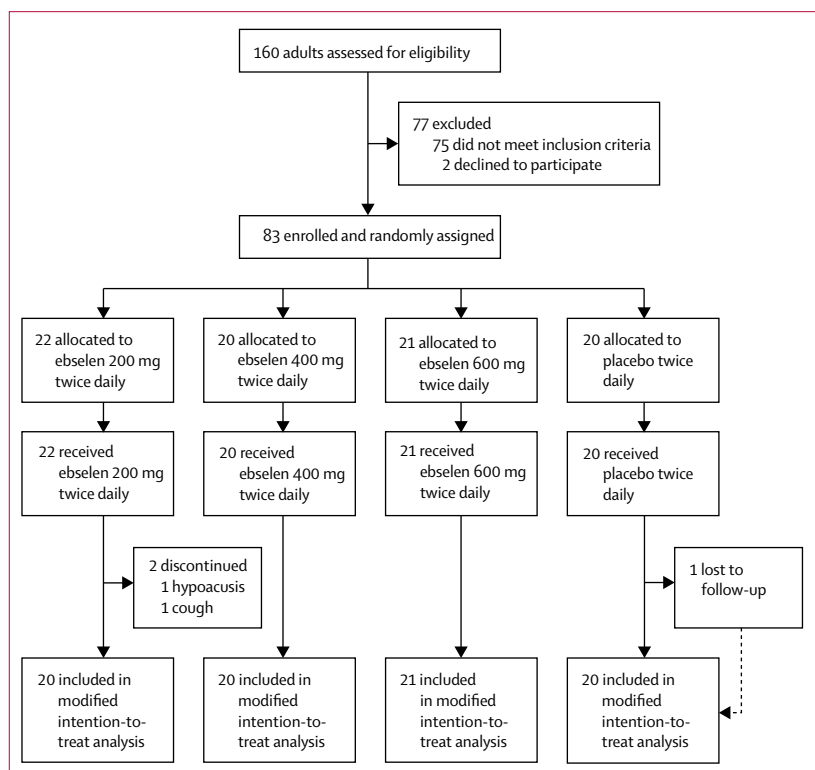
Systemic exposure to ebselen and its metabolites was measured in plasma samples taken at multiple time-points with a validated liquid chromatography–mass spectrometry method in compliance with Good Laboratory Practice guidance. The plasma concentrations from the peak and trough sampling were taken before and after the fifth oral dose when participants were expected to be at steady state. Plasma concentrations of selenium were also measured with a validated inductively coupled plasma mass spectrometry method, as previously described.<sup>21</sup> Plasma samples were obtained before dosing, during peak and trough sampling for ebselen and metabolites, and 5 days after the last scheduled dose. Haematological tests, serum chemistry tests (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin [total and direct], glucose, blood urea nitrogen, calcium, carbon dioxide [bicarbonate], chloride, serum creatinine,  $\gamma$ -glutamyl transferase, lactate dehydrogenase, magnesium, phosphate, potassium, sodium, total serum protein, and uric acid), and chest radiographs were done before, during, and after exposure to study drug. We compared all serial laboratory and radiographic findings, before and after dosing, between the ebselen groups and the placebo group.

## Outcomes

The primary outcome was mean TTS (difference between baseline hearing threshold and the post-noise hearing threshold in dB) at 4 kHz, determined 15 min after the calibrated sound challenge; we deemed a reduction of 50% in an ebselen group compared with the placebo group to be clinically relevant, on the basis of our clinical experience. The secondary outcome measure was a 50% reduction in mean TTS averaged across 3, 4, and 6 kHz, determined at 15 min after the calibrated sound challenge, in an ebselen group compared with the placebo group. Pharmacokinetic and safety outcomes were also assessed.

## Statistical analysis

On the basis of a previous observational study in which the mean TTS at 4 kHz measured 15 min after calibrated sound challenge was 6·3 dB,<sup>10</sup> and this interventional



**Figure 1: Trial profile**

Two participants randomly assigned to the 200 mg ebselen group did not receive the calibrated sound challenge, and were discontinued from further analysis because a noise-induced threshold shift could not be determined. The modified intention-to-treat analysis included all participants who received the calibrated sound challenge and at least one dose of study drug.

study's goal to reduce TTS severity by a clinically relevant difference of at least 50% between the placebo group and an ebselen dose group, a sample size of 20 participants per treatment group was calculated to be sufficient to allow confidence in rejecting the type 1 error ( $\alpha=0.05$ ) with 80% power ( $1-\beta$ ) for the primary outcome measure.

A mean TTS value was calculated per treatment group and analysed by a mixed-effects multiple repeated measures method using triply repeated measurements (ears, frequencies, and times) for each participant. The model had fixed effects for treatment, ear, frequency, and time; all two-way interactions and the three-way interaction treatment-by-frequency-by-time; plus a random effect for participant, nested within treatment. The covariance structure among the repeated measurements for a participant was assumed to be common across participants and modelled as the Kronecker product of an unstructured covariance ( $16 \times 16$ ) for ears  $\times$  frequency levels, and a first-order autoregressive covariance matrix for time. This analysis produced a variance measure of SE, which we have presented alongside the mean values.

Changes to the statistical analysis plan were made after database lock but before the statistical analysis was completed to broaden the analysis of TTS severity (mean threshold shift across 4, 6, and 8 kHz and across all tested frequencies from 0.25 kHz to 8 kHz), to determine the proportion of participants with a TTS of greater than or equal to 10 dB (significant threshold shift [STS]) at any frequency over time between 15 min and 3.25 h after the calibrated sound challenge, and the number of participants with return to baseline hearing (within 4 dB) in both ears at each timepoint after the calibrated sound challenge. An STS is judged to be clinically relevant and has been recommended as an analysis in clinical studies of noise-induced hearing loss.<sup>22</sup> Statistical comparisons were made between each ebselen dose group and the placebo group.

The analysis of participants with STS was done with the exact Cochran Armitage trend test at each timepoint, and a separate Fisher's exact test comparing the placebo group versus each ebselen dose group within a timepoint. In the return to baseline hearing analysis, pairwise comparisons were made with the Wilcoxon test and Bonferroni's adjustment to control for multiplicity with the placebo group versus an ebselen group.

All randomised participants who received the calibrated sound challenge and at least one dose of study drug were included in the efficacy analyses (modified intention-to-treat population). The mixed-effects multiple repeated measures method that was used for the efficacy analyses did not require complete data for each participant, so long as the data that were missing (either due to data collection or participant drop-out) were missing at random. All randomised participants were included in the safety analyses. All analyses were done with SAS software, version 9.3.

This trial is registered with ClinicalTrials.gov, number NCT01444846.

### Role of the funding source

The funder of the study was involved in the study design, data collection, data analysis, data interpretation, and writing of the report. JK, PJA, EDL, CGLP had full access to all the data in the study, and the corresponding author (JK) had final responsibility for the decision to submit for publication.

### Results

160 participants (80 men and 80 women) gave consent and completed at least part of the initial screening visit (figure 1). 83 participants (43 men and 40 women) met the inclusion criteria and were enrolled in the study between Jan 24, 2013, and March 11, 2014. Two participants in the 200 mg ebselen group were discontinued from the study before the calibrated sound challenge because they no longer met the inclusion and exclusion criteria. One participant in the placebo group did not return for the final scheduled visit. Therefore, 80 participants completed the study. Demographic and baseline characteristics are summarised in table 1. Baseline hearing thresholds were similar in participants in the placebo and ebselen groups (table 1). 81 participants completed the calibrated sound challenge and received either active or placebo treatment and are included in the efficacy analyses. Of these 81 participants, 77 participants completed the trial per protocol (all patients who received ebselen, underwent the calibrated sound

	200 mg ebselen (n=22)	400 mg ebselen (n=20)	600 mg ebselen (n=21)	Placebo (n=20)
<b>Age (years)</b>				
Mean (SD)	20.8 (1.60)	20.6 (1.27)	20.0 (0.89)	21.4 (2.23)
Median (range)	21.0 (19–25)	20.0 (19–23)	20.0 (18–21)	21.0 (18–26)
<b>Sex</b>				
Women	9 (41%)	9 (45%)	9 (43%)	13 (65%)
Men	13 (59%)	11 (55%)	12 (57%)	7 (35%)
<b>Ethnicity</b>				
Hispanic or Latino	2 (9%)	4 (20%)	3 (14%)	3 (15%)
Not Hispanic or Latino	20 (91%)	16 (80%)	18 (86%)	17 (85%)
<b>Race*</b>				
White	17 (77%)	16 (80%)	18 (86%)	16 (80%)
Black or African American	3 (14%)	3 (15%)	2 (10%)	1 (5%)
Asian	2 (9%)	2 (10%)	1 (5%)	3 (15%)
Unknown	1 (5%)	1 (5%)	0 (0)	0 (0)
<b>Baseline threshold (dB HL)</b>				
4 kHz	2.05 (0.98)	4.55 (1.03)	4.48 (1.00)	2.65 (1.03)
Mean of 3, 4, and 6 kHz	2.41 (0.80)	4.23 (0.84)	4.40 (0.82)	3.28 (0.84)
Mean of 4, 6, and 8 kHz	0.67 (0.85)	2.78 (0.89)	2.67 (0.87)	1.90 (0.89)
Mean of all frequencies (0.25–8 Hz)	3.60 (0.66)	4.42 (0.69)	4.17 (0.67)	3.95 (0.69)
Data are n (%) or mean (SE) unless otherwise stated. HL=hearing level. *Study participants could select more than one race category.				
<b>Table 1: Baseline demographic and clinical characteristics</b>				



	Mean threshold shift (dB)	Difference (treatment–placebo; dB)	SE*	95% CI	p value
<b>Modified intention-to-treat analysis</b>					
<b>4 kHz</b>					
600 mg ebselen	3.81	–0.27	0.90	–2.03 to 1.50	0.7659
400 mg ebselen	1.32	–2.75	0.91	–4.54 to –0.97	0.0025
200 mg ebselen	3.23	–0.84	0.91	–2.63 to 0.94	0.3542
Placebo	4.07	..	..	..	..
<b>Average of 3, 4, and 6 kHz</b>					
600 mg ebselen	2.87	–0.37	0.67	–1.68 to 0.94	0.5794
400 mg ebselen	0.82	–2.42	0.68	–3.74 to –1.09	0.0004
200 mg ebselen	2.22	–1.02	0.68	–2.35 to 0.31	0.1321
Placebo	3.24	..	..	..	..
<b>Average of 4, 6, and 8 kHz</b>					
600 mg ebselen	1.66	–1.35	0.65	–2.63 to –0.08	0.0378
400 mg ebselen	0.86	–2.15	0.66	–3.44 to –0.85	0.0011
200 mg ebselen	1.30	–1.71	0.66	–3.01 to –0.42	0.0094
Placebo	3.01	..	..	..	..
<b>Average of all frequencies</b>					
600 mg ebselen	1.62	–0.98	0.52	–2.00 to 0.05	0.0620
400 mg ebselen	0.48	–2.11	0.53	–3.15 to –1.07	<0.0001
200 mg ebselen	1.28	–1.31	0.53	–2.35 to –0.27	0.0134
Placebo	2.59	..	..	..	..
<b>Per-protocol analysis</b>					
<b>4 kHz</b>					
600 mg ebselen	3.69	–0.40	0.93	–2.22 to 1.41	0.6638
400 mg ebselen	1.30	–2.79	0.90	–4.56 to –1.03	0.0020
200 mg ebselen	3.35	–0.74	0.91	–2.53 to 1.05	0.4171
Placebo	4.09	..	..	..	..
<b>Average of 3, 4, and 6 kHz</b>					
600 mg ebselen	2.59	–0.62	0.68	–1.95 to 0.71	0.3580
400 mg ebselen	0.85	–2.37	0.66	–3.66 to –1.07	0.0003
200 mg ebselen	2.33	–0.89	0.67	–2.20 to 0.42	0.1829
Placebo	3.21	..	..	..	..
<b>Average of 4, 6, and 8 kHz</b>					
600 mg ebselen	1.35	–1.62	0.65	–2.90 to –0.34	0.0133
400 mg ebselen	0.88	–2.09	0.64	–3.34 to –0.84	0.0010
200 mg ebselen	1.48	–1.49	0.64	–2.75 to –0.23	0.0209
Placebo	2.97	..	..	..	..
<b>Average of all frequencies</b>					
600 mg ebselen	1.44	–1.14	0.53	–2.17 to –0.11	0.0297
400 mg ebselen	0.48	–2.09	0.51	–3.10 to –1.09	<0.0001
200 mg ebselen	1.44	–1.14	0.52	–2.15 to –0.12	0.0285
Placebo	2.58	..	..	..	..

Mean threshold shift (dB) between the baseline hearing threshold and the post-noise hearing threshold measured 15 min after calibrated sound challenge determined at one (primary) and multiple (secondary) frequencies. Significance determined with a mixed-effects multiple repeated measures analysis. \*This is the SE of the mean difference between treatment and placebo.

**Table 2: Primary and secondary outcomes**

challenge, and attended all follow-up visits) and are included in the per-protocol analysis (ebselen 200 mg n=19, 400 mg n=20, 600 mg n=18, placebo n=20). All 83 enrolled participants are included in the safety analyses.

At 15 min post noise, mean TTS at 4 kHz was 4.07 dB (SE 0.90) in the placebo group compared with 1.32 dB (0.91) in the 400 mg ebselen group, representing a reduction of 68% (modified intention-to-treat population; difference –2.75 dB, 95% CI –4.54 to –0.97; p=0.0025; table 2). Compared with placebo, mean TTS at 4 kHz was reduced, although non-significantly, by 21% in the 200 mg ebselen group and by 7% in the 600 mg ebselen group (table 2). TTS across 3, 4, and 6 kHz was a mean of 3.24 dB (SE 0.67) in the placebo group compared with 0.82 dB (0.68) in the 400 mg ebselen group (75% reduction; p=0.0004; table 2). Statistically significant or clinically relevant (≥50%) reductions in TTS severity were seen in all three ebselen groups (200 mg, 400 mg, and 600 mg) compared with placebo averaged across 4, 6, and 8 kHz in the modified intention-to-treat analysis and across 4, 6, and 8 kHz and all tested frequencies (0.25–8 kHz) in the per-protocol analysis. Seven (58%) of the 12 treatment versus placebo comparisons in the modified intention-to-treat analysis and eight (67%) of the 12 comparisons in the per-protocol analysis were significant.

At 15 min post noise, the proportion of participants with STS was 60% (12 of 20 participants) in the placebo group compared with 20–30% (four to six of 20) in the ebselen groups, with the lowest proportion in the 600 mg group (p=0.0203), followed by the 200 mg group (p=0.0268) and the 400 mg group (p=0.0555; modified intention-to-treat population; figure 2). At 3.25 h after the calibrated sound challenge, the proportion of participants with STS was 35% (seven of 20) in the placebo group, whereas the proportion in the ebselen groups was as low as 5% (one of 20). The exact Cochran Armitage trend test for each timepoint was found to be p=0.0188 at 15 min and p=0.0309 at 3.25 h after calibrated sound challenge in the modified intention-to-treat analysis, and p=0.0183 at 15 min and p=0.0119 at 3.25 h in the per-protocol analysis. A separate Fisher's exact test was done to compare the placebo group with each ebselen dose group, at each timepoint after calibrated sound challenge. In the modified intention-to-treat analysis, six of the 12 comparisons were significant, whereas in the per-protocol analysis, seven of 12 comparisons were significant.

For most participants, return to baseline hearing occurred within 24 h after calibrated sound challenge irrespective of treatment (figure 3). All ebselen dose groups had a greater number of participants (19 [95%] to 20 [100%] of 20) who returned to baseline hearing within 3.25 h of the calibrated sound challenge than did the placebo group (17 [85%] of 20). However, no significant differences with regard to time taken to return to baseline hearing were seen between any ebselen group and the placebo group using the Wilcoxon test and Bonferroni's adjustment to control for multiplicity. Six (8%) of the 80 participants who completed the study returned for an additional hearing test 1 week after clinic

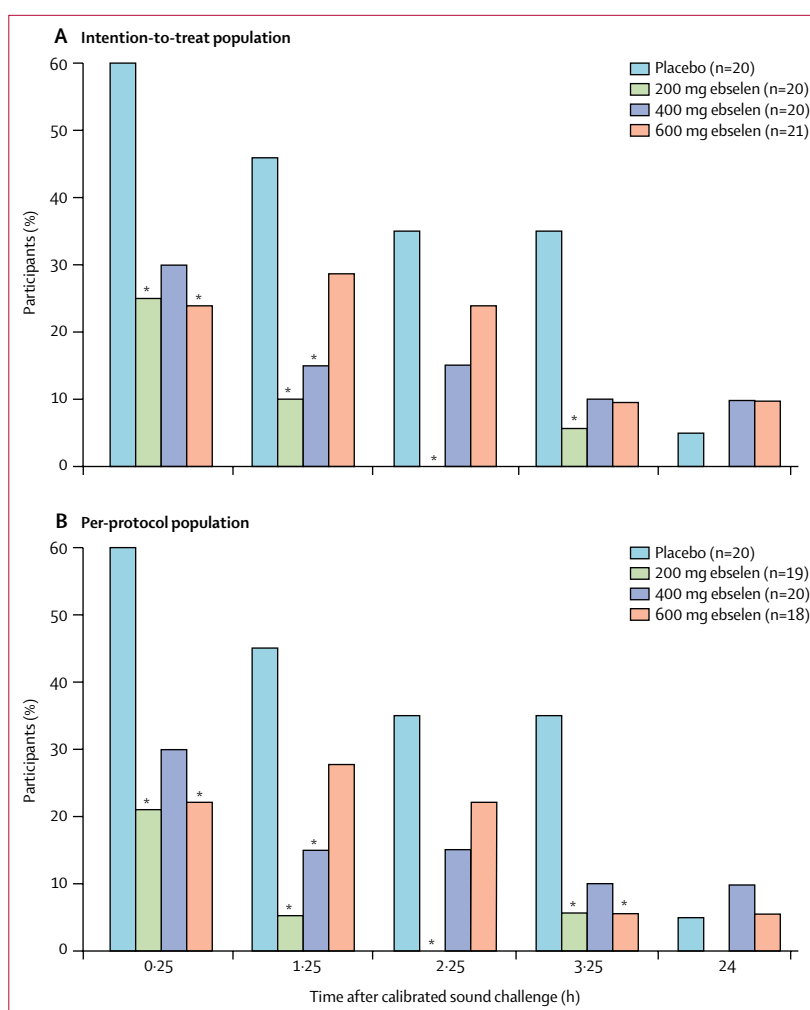
visit 4 (2 weeks after the calibrated sound challenge; one [5%] in the 200 mg ebselen group, one [5%] in the 400 mg ebselen group, and four [20%] in the placebo group). The reason for the additional re-test was that 1 week after calibrated sound challenge the hearing threshold of these participants was at least 6 dB higher than their baseline threshold at one or more tested frequencies. Return to baseline hearing was reported within 1 week in all but one (1%) of the 80 participants at the time of their final test (either 1 week after the calibrated sound challenge, or 2 weeks after [in the six patients who were re-tested]). The screening and baseline hearing thresholds of the one participant whose hearing had not returned to baseline, who was in the placebo group, were highly variable ( $>4$  dB), and the thresholds assessed after the calibrated sound challenge showed improvements in some low frequency hearing (from 6 dB to 10 dB) compared with baseline. Mean hearing thresholds assessed before (screening and baseline) and after (24 h and 1 week) the calibrated sound challenge across all tested frequencies (0.25–8 kHz) for each group are shown in figure 4.

Ebselen concentration in blood was determined by a single peak and trough measurement taken before and 2 h after the fifth oral dose. The mean trough concentration of ebselen ( $C_{\min}$ ) ranged from 0.082 ng/mL (SD 1.80) for the 200 mg dose to 0.179 ng/mL (1.59) for the 600 mg dose. The mean peak concentration of ebselen ( $C_{\max}$ ) ranged from 0.148 ng/mL (1.64) for the 200 mg dose to 0.372 ng/mL (1.61) for the 600 mg dose. The predominant metabolite of ebselen in plasma was the 2-glucuronyl selenobenzanilide. Combined plasma ebselen and metabolite concentrations showed a positive correlation with plasma selenium concentrations in all ebselen dose groups ( $R^2=0.528$ ).

Repeat analysis of histories, physical examinations, vital signs, haematology (full blood count), serum chemistry tests, and chest radiographs were similar between the ebselen groups and the placebo group, and no significant differences were noted (data not shown).

## Discussion

Our findings show that ebselen was safe and effective in preventing acute noise-induced hearing loss in healthy adults. In terms of safety, no significant differences were seen in any haematological, serum chemistry, or radiological assessments between the ebselen groups and the placebo group. These results are consistent with findings of the previous phase 1 clinical trial, in which no adverse events related to drug were reported.<sup>21</sup> In this phase 2 trial, treatment with ebselen 400 mg twice daily resulted in a clinically relevant and statistically significant reduction in TTS severity compared with placebo. This finding was not seen for the 200 mg or 600 mg doses. However, significant reductions in TTS severity across 4, 6, and 8 kHz were seen in all ebselen groups (200 mg, 400 mg, and 600 mg), and across all tested frequencies for the 400 mg and 600 mg groups. A significant reduction in the proportion of participants with an STS

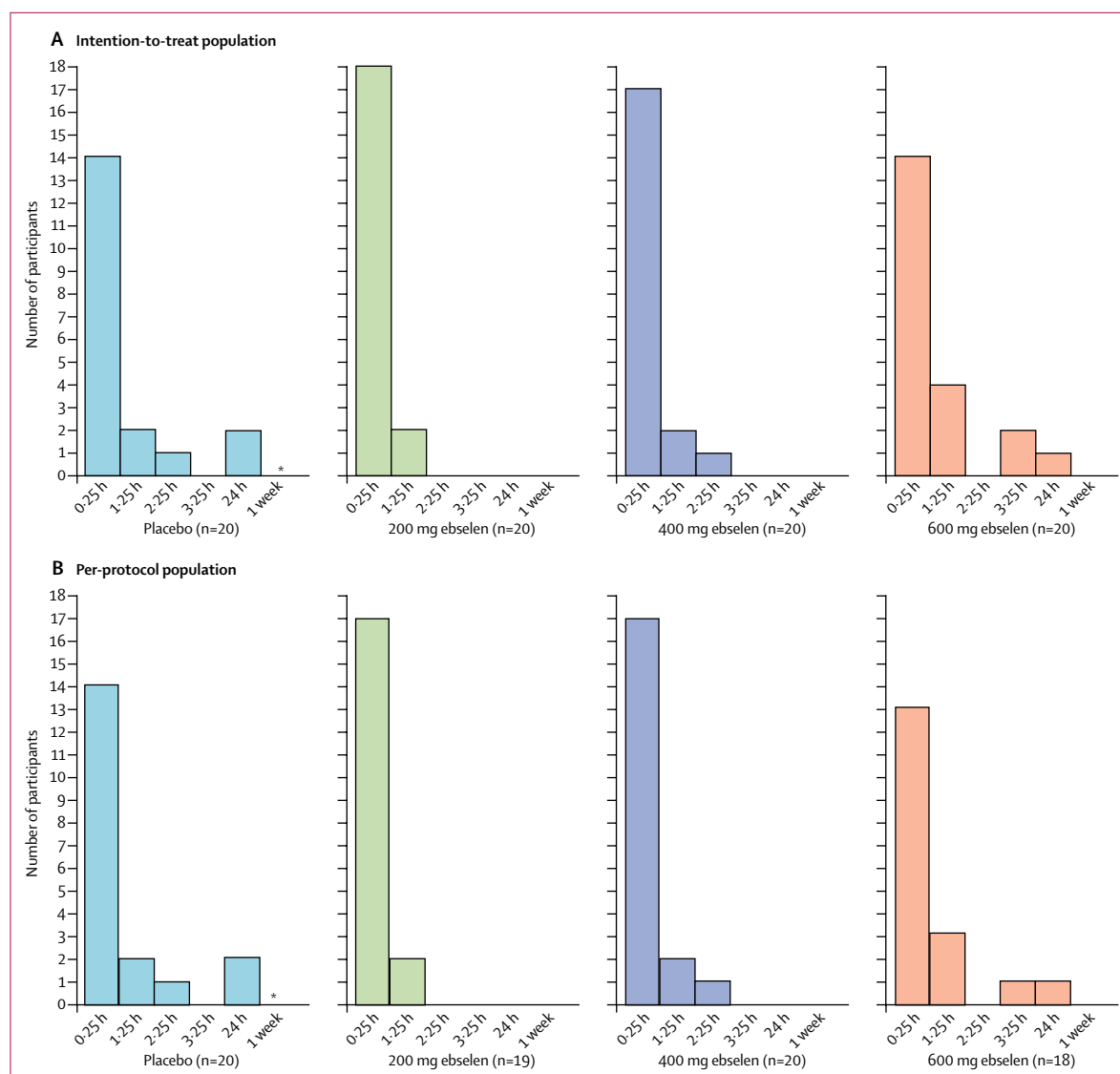


**Figure 2: Proportion of participants with a clinically significant threshold shift induced by the calibrated sound challenge**

A significant threshold shift is defined as a temporary threshold shift of greater than or equal to 10 dB. \* $p<0.05$  (Fisher's exact test for placebo vs ebselen group).

was seen in the 200 mg and 600 mg ebselen groups compared with placebo, but not in the 400 mg group.

Limitations of our study include the length of pre-exposure dosing and the absence of a clear dose response in the primary and secondary outcome measures. First, all participants began treatment 2 days before the calibrated sound challenge. Therefore, additional studies are needed to determine whether ebselen treatment would exert a similar reduction in TTS severity ( $\geq 50\%$  at 15 min after calibrated sound challenge) if dosing began closer to the onset of noise exposure. To achieve steady-state concentrations of ebselen in human beings, oral dosing 24 h before a significant noise exposure might be required. However, a single dose of ebselen given 1 h before an intense noise exposure prevented TTS in adult guinea pigs.<sup>19</sup> Second, the absence of a clear dose response to ebselen treatment was reported in guinea pigs, in which 10 mg/kg seemed to be better than 30 mg/kg in



**Figure 3: Number of participants returning to baseline hearing thresholds (within 4 dB) in both ears by timepoint after calibrated sound challenge**

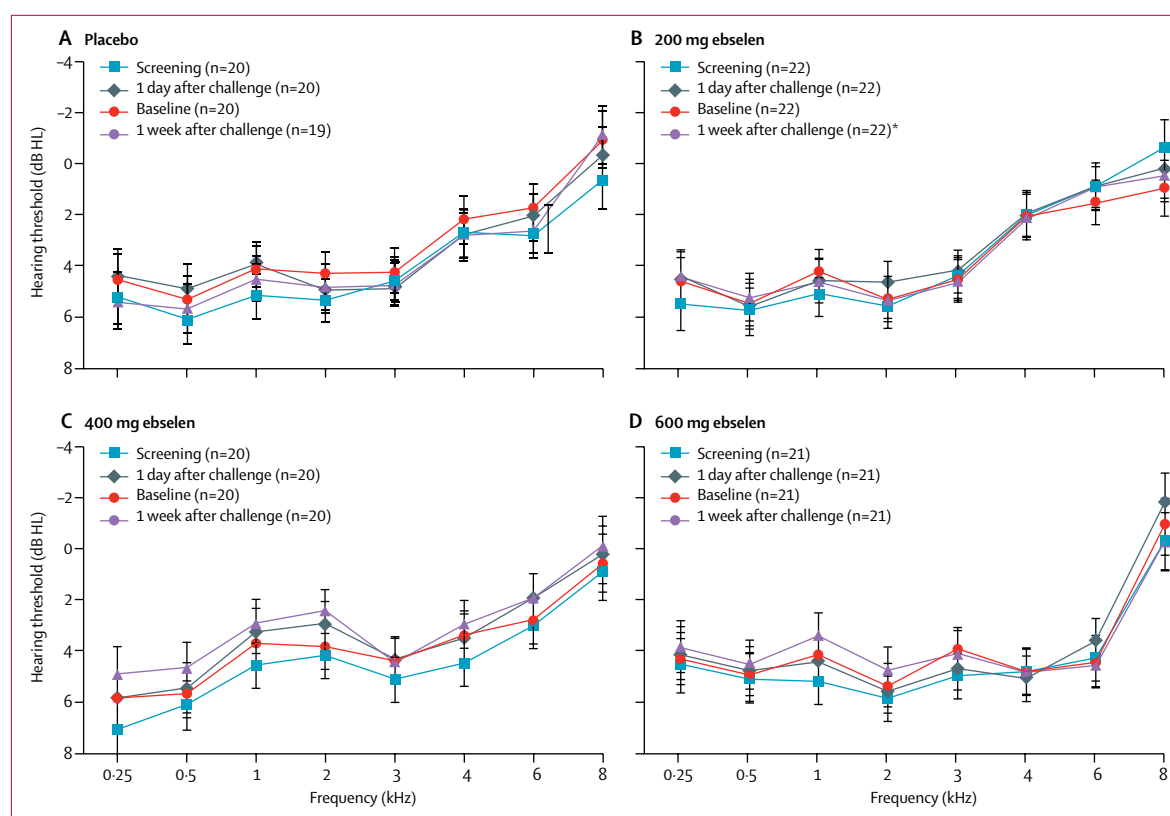
No significant differences were seen between any ebselen group and the placebo group using the Wilcoxon test and Bonferroni's adjustment to control for multiplicity. \*One (5%) participant in the placebo group had not returned to within 4 dB of their baseline hearing 1 week after the calibrated sound challenge, and they were subsequently lost to follow-up.

preventing a noise-induced PTS.<sup>17</sup> Whether ebselen treatment would prevent a noise-induced PTS or show a similar lack of dose response in people is not clear, since that endpoint was not assessed in this study. Additional testing might be necessary to identify an optimum dose for the prevention of acute noise-induced hearing loss, whether TTS or PTS. Third, the small sample size of this study might have led to imbalance in the enrolment of so-called tough versus tender ears between the treatment groups, resulting in the absence of dose response. However, the baseline hearing thresholds across the three ebselen dose groups and the placebo group did not differ significantly, suggesting that randomisation to treatment groups was unbiased. Additionally, participants with

baseline hearing loss of more than 24 dB HL were excluded from enrolment in this study. Family history of noise-induced hearing loss and an individual's previous noise exposure were not considered in this initial study, but could be considered in future studies. For these reasons, we must acknowledge the possibility of a type 1 error in the conduct and analysis of this phase 2 trial.

Although limited, the hearing protection demonstrated in this initial human study correlates with the otoprotective findings from several studies in animal models, in which detailed anatomical and physiological assessments were possible. In adult rats, ebselen treatment resulted in decreases in TTS (at 24 h) and PTS (at 3 weeks) after noise exposure.<sup>18</sup> Significant reductions in hearing loss were





**Figure 4:** Mean hearing thresholds before and after calibrated sound challenge at all test frequencies (0.25–8 kHz)

Error bars show SE. HL=hearing level. \*Two of 22 participants did not undergo the calibrated sound challenge, but were included here because they attended the 1 week follow-up visit and were part of the safety population.

noted after single and repeat noise exposures (14 dB ebselen vs 42 dB control), a 68% decrease in TTS severity. The PTS in ebselen-treated animals was almost fully resolved, whereas the PTS in controls was 14 dB. Additionally, outer hair cell loss was reduced by 62% in ebselen-treated animals compared with control animals. In an independent study of the same animal model of noise-induced hearing loss, ebselen reduced noise-induced immunostaining for reactive oxygen and nitrogen species, and preserved the survival of several cell types within the spiral ligament, stria vascularis, and spiral ganglia neurons.<sup>20</sup> Finally, in adult guinea pigs, a single oral dose of ebselen (10 mg/kg) 1 h before the noise exposure prevented a robust TTS (25–45 dB) and afferent dendrite swelling at all frequencies tested (2, 4, 8, and 16 kHz). The electrophysiological and electron microscopy data detailed in this independent study further substantiated the physiological and anatomical changes in the cochlea after a robust TTS, and the benefit of ebselen treatment immediately before an intense noise exposure.

We previously identified that GPx1 is highly expressed in specific sensory, non-sensory, and neuronal cell types in the cochlea that are involved in acute noise-induced hearing loss. In other animal models of acute sensorineural hearing loss, ebselen significantly reduced the ototoxicity induced by cisplatin treatment.<sup>23</sup> Prevention

of cisplatin-induced ototoxicity by ebselen seems to be mediated through the preservation of intracellular glutathione<sup>24</sup> and the activation of the Nrf2 transcription factor and other cytoprotective genes.<sup>23</sup> Furthermore, susceptibility to chronic noise-induced hearing loss in people might also be associated with an SNP in *GPX1* versus SNPs in *SOD1* or *SOD2*.<sup>8</sup> Taken together, these findings support the importance of GPx1 activity in preventing or treating noise-induced hearing loss and potentially ototoxicity. Our findings suggest the potential for ebselen in the prevention and treatment of chronic noise-induced hearing loss, age-related hearing loss, and ototoxicity.

Notably, substantial variability in TTS between placebo-treated and untreated study participants has been reported in the two previous studies of calibrated sound challenge.<sup>10,11</sup> To estimate the noise dose during the calibrated sound challenge, our recorded sound levels are converted from sound coupler levels to a free field equivalent (FFE), based on the average ear, using Occupational Safety and Health Administration (OSHA) criteria.<sup>25</sup> However, when FFE levels are measured in individual ears, individual specific FFEs are typically 5–15 dB less than the measured in-ear level because of variability in the resonance of an individual's ear canal.<sup>10</sup> In other words, whereas the sound delivered from the

insert earphone is carefully controlled across participants, the sound reaching the eardrum differs substantially as a function of an individual's ear canal volume and resonance. To be conservative, the 100 dBA coupler level was adjusted down by 5 dB, the smallest FFE value yielding an estimated FFE exposure level of 95 dBA. Since the duration of the calibrated sound challenge was held constant for 4 h, the challenge provides up to 100% of the recommended daily permissible exposure level described by OSHA. In a workplace setting, OSHA states that the repetition of these permissible exposure levels on a daily basis has the potential to result in material hearing loss during the course of a 40-year career.

A noise-induced TTS that has not returned to baseline levels within 2 weeks is at risk for becoming a PTS, and a TTS that has not resolved to baseline within 30 days of noise exposure is assumed to be a PTS.<sup>12</sup> Animal data have shown a permanent neural denervation after a 40–50 dB TTS measured 24 h post noise exposure in the absence of a PTS.<sup>14–16</sup> However, additional studies in both developing (6 weeks) and adult (16–18 weeks) mice show that a 20–30 dB TTS measured 24 h post noise was not neuropathic, even when the animals were followed up for extended time periods.<sup>26,27</sup> In our study, one of the 81 participants exposed to the calibrated sound challenge had not returned to within 4 dB of his or her baseline hearing in both ears 1 week after the calibrated sound challenge. In this participant, who was in the placebo group, the TTS ranged from –10 dB to 6 dB across all test frequencies in both ears. The threshold improvements of 6–10 dB (at 1, 2, and 3 kHz), should be taken into account when interpreting the threshold reductions of 6 dB (at 6 kHz and 8 kHz). The relevance of these data and the future determination of risk criteria are of great interest.

The mean TTS recorded here (4.1 dB at 4 kHz, 15 min after calibrated sound challenge) was smaller than that reported in the initial observational study (6.3 dB at 4 kHz, 15 min after calibrated sound challenge) in similar study participants enrolled at the same clinical site.<sup>10</sup> This reduction in TTS severity reduces the power to detect an otoprotective effect of ebselen treatment in a small sample size, and might have contributed to the absence of a dose response in the primary and secondary outcome measures. A study in 70 adults who were pretreated with an oral supplement containing vitamins A, C, E, and magnesium for 4 days and exposed to a calibrated sound challenge also showed a 4 dB TTS at 4 kHz, 15 min after the calibrated sound challenge.<sup>11</sup> In that study, no significant improvements in TTS were seen in participants in the active group compared with the placebo group.<sup>11</sup> The outcome of that supplement study contrasts with the findings of this investigational new drug study.

Our findings support the continued assessment of ebselen for the prevention of noise-induced hearing loss (NCT02779192). Ebselen is under active clinical investigation in two other related inner ear diseases—

namely, for the prevention and treatment of ototoxicity (NCT01451853, NCT02819856) and Meniere's disease (NCT02603081). The importance of noise-induced TTS as a precursor to noise-induced PTS<sup>28</sup> has been detailed in occupational guidance for noise-induced hearing loss,<sup>29</sup> and noise-induced TTS has the potential to compromise mission success for service members in the armed forces.<sup>30</sup> In future studies, we plan to determine the safety and efficacy of ebselen in participants with greater baseline hearing loss who are at risk for progressive noise-induced hearing loss because of their occupational or recreational noise exposure.

#### Contributors

JK and CGLP contributed to the literature search, figures, study design, data analysis, data interpretation, and writing of the report. EL contributed to the literature search, study design, data analysis, data interpretation, and writing of the report. CS, SKG, and PJA contributed to data collection and analysis, data interpretation, and writing of the report. EDL contributed to the figures, study design, data analysis, data interpretation, and writing of the report.

#### Declaration of interests

JK and EDL are employees and stock owners of Sound Pharmaceuticals. EL, CS, SKG, PJA, and CGLP received funding from Sound Pharmaceuticals to do this research. CGLP reports contract support from Edison Pharmaceuticals, outside the submitted work. CGLP is a co-inventor on patents awarded to the University of Florida (issued), and two patents awarded to the University of Michigan, both of which are licensed to Hearing Health Science. JK and EDL have a patent issued for ebselen and hearing loss.

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