Noise-Induced Hearing Disorders: Hidden and Not-So-Hidden Injuries, in partnership with American Auditory Society

Presenter: Colleen Le Prell, PhD

- [Christy] We welcome back Dr. Colleen Le Prell. Dr. Le Prell is out of the University of Texas at Dallas, and if you'd like to learn more about Dr. La Prell and her research, you can read about her course bio on the registration page, but at this time, I'm gonna hand the mic over to you, Dr. La Prell.

- Thank you, and thank you to everybody who is here, I hope that today's webinar will help you get back into the swing of things, thinking about audiology and science. These are my disclosures, in particular, I'll just point to having received support from the NIH-NIDCD, the Department of Defense, and also from industry for support of clinical trials, which I'll be talking about today. And these are our learning outcomes for today. What we're going to talk about today is information related to cochlear synaptopathy, and some of the functional deficits that are thought to be a potential consequence of cochlear synaptopathy. Probably a lot of you have heard the phrase "hidden hearing loss" previously, which has been used to refer to many of the elements we'll be talking about today, and so I will begin by providing some terms and definitions that are related to hidden hearing loss, to get us started.

When we talk about hidden hearing loss, this really was initiated from some paradigm-changing research from Kujawa and Liberman, in 2009, they documented a noise-induced decrease in Wave I amplitude in the presence of normal ABR thresholds with the loss of cochlear synapses with the hair cells, after recovery from noise exposure. And one of the reasons this was such a important finding is it suggested that there is a permanent pathology that can be induced by noise exposure, which isn't captured through threshold measurements or outer hair cell counts, which are the two things that we've really relied on in all of the animal research for many, many, many, many years, going back to the earliest days of noise injury research.

And in 2011, Schaette and McAlpine termed this decreased Wave I amplitude in the presence of normal thresholds as hidden hearing loss. And when you hear people use the phrase "hidden hearing loss", sometimes it's used to refer to the cochlear synaptopathy, that loss of synapses between the inner hair cells and the afferent fibers, sometimes it's used to refer to this decreased Wave I present, this decreased Wave I amplitude in the presence of normal thresholds, and sometimes it's used to refer to the superthreshold deficits that we think might be a consequence of the cochlear synaptopathy. So tinnitus, hyperacusis, and hearing and noise difficulties are three of the major functional deficits that have been thought to be a consequence of cochlear synaptopathy, and there has been research with human participants and research in animal models on a steady basis since 2009, since those first findings came out, to really try and understand this phenomena, and really try and understand the functional consequences.

And in general, I'm going to try and use the specific terms to refer to either the pathology, the electrophysiology, or the functional consequences, rather than using hidden hearing loss, since that has become somewhat of a catchall phrase for these three very different elements, the pathology, the electrophysiology, and the function. So when we talk about the deficits that are potentially related to synaptopathy, one of the major challenges is that some of these deficits can also be due to the loss of outer hair cells. So if we think about what do we usually measure in clinical research, audiology research, hearing research, threshold deficits are one of the things that are very heavily focused on, and hearing loss threshold deficits can be measured using the audiogram in humans, you can also collect an audiogram in trained animal participants, but particularly in animal experiments, you'll see ABR thresholds or DPOAE thresholds used in place of a behavioral audiogram.

And depending what causes the threshold elevation, we might call it NIHL, if it's noise that induces the hearing loss, age-related hearing loss, ARHL, if it's related to an

ototoxic drug like cisplatin, or the immuno-glycoside antibiotics, we might refer to it as DIHL for drug-induced hearing loss, so those are all audiogram changes that are typically thought to be associated with the loss of outer hair cells, they're the clinical gold standard, and really they've been the primary outcome measure in the vast majority of clinical trials to date. And we'll talk about clinical trials later in this presentation. Now, superthreshold deficits are of a lot of interest for multiple reasons, and hearing a noise in particular has emerged as a major focus of research, in part because of the speculation that cochlear synaptopathy might be a cause of hearing and noise deficits.

Obviously, there have been many years of research on tinnitus, including both efforts to understand outer hair cell pathology that results in tinnitus, and also neural pathology that results in tinnitus, there's many causes of tinnitus. Hyperacusis is something that is not as well-understood, so collectively, we can refer to these superthreshold deficits as noise-induced hearing disorders, or drug-induced hearing disorders, if they're caused by a ototoxic drug agent, but we don't have really good strategies for saying this, "If you observe this, then the patient has outer hair cell loss", or "If you observe this, then the patient has afferent loss" because both outer hair cell loss and afferent loss have the potential to cause hearing and noise deficits, and have the potential to cause tinnitus.

So these have not been as well-represented in clinical trials, because they have various etiologies, and we simply have less agreement on how to measure these different phenomena. We have many tinnitus surveys, we have many hearing and noise tests, we have less standardization of those procedures. So now that we've talked a little bit about some of the basics of the different pieces that go into hearing loss, I wanna talk with you specifically about noise-induced cochlear pathology, and all of the things that noise does to the cochlea, including its pathology to the afferents. When we think about noise exposure, there's really a continuum with increasing noise causing

increasing pathology. If you have a shorter or less intense noise, that's typically going to result in a more minor and reversible kind of pathology, that can include neural swelling, swelling of the afferents below the hair cells, it can include damage, stereocilia, the hair cells might be swollen or misshapen, there will typically be a temporary change in thresholds, and most of that pathology will recover in the case of a very large TTS, as we now know, there can be permanent consequences as well with the disconnection of the inner hair cells to the afferents, with the loss of those synaptic connections.

As you have increasing noise exposure, you start to have pathology of the outer hair cells, so the outer hair cells, I'm sure you're all very familiar with, and use distortion product otoacoustic emissions to monitor outer hair cell integrity, the outer hair cells are, of course, the cochlear amplifier, they provide gain to the traveling wave at lower sound pressure levels, and they give you about 40 dB of benefits. So if you have 100% loss of the outer hair cells, you'll have about a 40 dB threshold shift, because you lose all of that cochlear amplification. There will still be a traveling wave that's generated with the mechanical energy, it still has the ability to stimulate the inner hair cells, and so you don't have a profound deafness with loss of the outer hair cells, you lose that gain function.

If the inner hair cells are damaged, you can lose a lot of inner hair cells before you have a threshold deficit, some of the work with carboplatin in chinchillas, which is a really unique paradigm, because carboplatin is selectively toxic to the inner hair cells in the chinchilla, in those animals, you have a selective inner hair cell loss and you can lose about 80% of the inner hair cells before you have threshold changes. Above 80% loss of the inner hair cells, or if you have a significant loss of the auditory nerve fibers, again, a profound loss, there's a lot of redundancy, so you can lose quite a few auditory nerve fibers before you have a threshold loss, then you'll get into those more severe hearing losses.

There's also the potential for mechanical damage, particularly with impulse noise, if there's a sudden and profound jump in sound exposure, high-level impulse noise, you can have a breach of the reticular lamina, and that allows mixing of the perilymph that is located in the scala vestibuli and the scala tympani with the endolymph that's in scala media, which is toxic to the hair cells, and can cause a very profound jump in the amount of permanent threshold shift that's observed after noise exposure, when you reach levels that breach the reticular lamina. Those are the kinds of pathology that most people are probably relatively familiar with, what you might be less familiar with is the active metabolic cell death that occurs, as well, and so in addition to the potential for some cells to be damaged due to the mechanical energy introduced by noise exposure, there's an ongoing stress reaction, an ongoing free radical formation in the inner ear, and free radicals will accumulate over multiple days, they reach their peak at about seven to 10 days post-noise exposure, and it turns out that there's a very tight correlation between when you see these dramatic increases in free radical formation, and when hair cells are dying.

And so that is one of the reasons that antioxidants have been of a lot of interest, because they target the free radical formation, and if you intervene even post-noise exposure with agents that ameliorate the free radical formation in the cochlea, you can prevent a lot of the outer hair cell death that would otherwise occur. And this has been shown with a number of different antioxidant agents, there are probably more than 100 antioxidants that have been investigated in animal models, and the vast majority of them have shown at least some level of benefit. Just in terms of free radicals themselves, what they are, they're part of normal metabolism, so free radicals are part of the formation of cell energy, we consume glucose, sugars, we breathe in oxygen, this gets broken down in cells, and turned into cellular energy, ATP, and as you have this long series of chemical reactions through which the sugars and the oxygen are being converted, being metabolized to form ATP, you have electrons that essentially

get lost, these are oxygen molecules, or hydrogen molecules, or nitrogen molecules, they're charged electrons, and they're looking for something to bind to, and when they're not neutralized by binding with another molecule that has the opposite charge, then they can damage the cell membranes, so literally generate holes in the cell membranes surrounding the outer hair cells, they can damage the mitochondria, inside the hair cells, they can damage DNA inside the nuclei of the cells, and cell death can occur as a consequence of the damage to the membranes, the damage to the mitochondria, the damage to the nucleus, and the DNA.

Interestingly, these electrons that accumulate in the outer hair cells after noise exposure, they also accumulate after immuno-glycoside antibiotic administration, and they accumulate during cisplatin therapy, so a lot of the agents that have been studied with one form of pathology have been studied for multiple indications, so not only for noise injury, but also for drug-induced hearing loss, some have even been studied the attenuation, the slowing of age-related hearing loss, because of the role of free radicals in causing outer hair cell pathology, and all of these different injuries. So the maximum accumulation, as I said, of free radicals occurs about seven to 10 days post-noise exposure, and this has created a window of opportunity for therapeutic rescue.

In some of the research that we've done, the window is about three days, once you get out to five days post-noise, there wasn't any benefit of the antioxidant compounds that we used, but clearly, the earlier the better, pre-noise was the most effective in attenuating hearing loss, as you delayed the delivery from one hour to one day to three days post-noise, they become less and less effective. So the next thing that I want to talk about is some points of agreement on cochlear synaptophathy, and also some of the questions that labs around the world, including my own, are working on trying to answer, so one of the first things that has become really clear early on, there was a lot of concern that every TTS-inducing noise exposure would result in cochlear synaptopathy, and we know that that is not true, there are exposures that cause

temporary threshold shift that do not cause cochlear synaptopathy, what is less clear is what are the factors that dictate whether or not a TTS causes a cochlear synaptopathy.

One of the challenges is, it's not a simple relationship between the amount of temporary threshold shift, and whether a synaptic pathology occurs, you can have the exact same pathology, for example, a TTS of 20 dB at 20 Kilohertz in an animal model, but depending what happens at the higher frequencies, you may or may not get cochlear pathology at that 20-Kilohertz-frequency, in animals where there is a sloping increasing hearing loss with a louder or longer noise exposure, synaptopathy will be observed, in animals that have a very notched-like exposure, where you don't have a significant higher frequency hearing loss, there may be no synaptopathy at that frequency, and that comes from work from Kate Fernandez in Sharon Kujawa's lab.

So really elegant studies trying to probe the relationships between temporary threshold shift and cochlear synaptic pathology. The other finding that has observed is this sort of paradoxical finding, in which, as you get more and more permanent threshold shift, it actually appears to be protective in that you get decreasing synapse loss, as you have increasing permanent threshold shift, again, that's work from Kate Fernandez in Sharon Kujawa's lab. So there are some things that we don't understand about what I would call the damage-risk relationship, what is the relationship between the amount of noise exposure, the amount of temporary threshold shift, and the likelihood of cochlear synaptopathy at different frequencies. The other thing that's incomplete understood is vulnerability of humans.

There has been a lot of work in mouse models, and there has been some work done in several other rodent models, including the chinchilla, the Guinea pig, and the rat, and there has been a little bit of work in non-human primates, Rhesus macaques, in particular. And if you look at the mouse literature, so most of this is done in a CBA-CHA adult mouse, and most of it has been done with 100 db SPL octave band

noise for two hours, and if you increase that level by three dB, that TTS cochlear synaptopathic exposure becomes a PTS-inducing exposure, if you decrease the noise level by three dB, then you no longer see cochlear synaptopathy, even though you still see a temporary threshold shift.

So it's a very narrow region, where increasing or decreasing the level by three dB will go from no pathology to a PTS pathology, rather than this selective cochlear synaptopathic injury. If you look in the chinchilla, their vulnerability is very similar to the mouse, 98 to 99 dB SPL octave band noise for two hours induces a selective cochlear synaptopathy, increasing the noise by two dB increased the pathology to a permanent threshold shift. In the Guinea pig, it takes more noise, 160 db SPL octave band noise for two hours, if you increase the level by three dB, just as in the mouse, you move from a synaptopathic injury to an injury that causes outer hair cell loss, and permanent threshold shift.

The rat that takes a little bit more noise than in the Guinea pig, 190 dB SPL octave band noise for two hours will induce a selective synaptopathic injury, if you drop down three db to the 106 dB exposure used in Guinea pigs, there's no synaptic injury. So across rodent models, you see this very narrow range of noise exposures, where you quickly transition from no pathology to synapse loss, to outer hair cell loss, and permanent pathology. In the Rhesus macaque, it takes quite a bit more noise to induce a selective cochlear synaptopathy, 108 dB SPL narrow band noise for four hours, this was a 50 Hertz noise band centered at two kilohertz, so a very tonal stimulus, very focused stimulus for four hours, or 120 dB SPL octave band noise for four hours in order to induce a temporary threshold shift and synapse loss.

So quite a bit more noise injury than was -- Quite a bit more noise than was required in the rodent models. And so there's a lot of questions about how this translates to humans, and in studies that have not found evidence of cochlear synaptopathy, it

doesn't necessarily mean that cochlear synaptopathy doesn't occur, but perhaps the exposures were not at the levels that are necessary to cause a noise-induced synaptopathy in humans. The studies that have shown the most compelling evidence of a synaptopathy like a phenotype in humans have been humans who are exposed to firearm noise exposure, and that's worked from Naomi Bramhall with veterans and service members, and also with civilian firearm users. Humans, I don't want to leave the suggestion that they're not at risk for cochlear synaptopathy, while we don't know where the vulnerability for noise-induced synapse loss begins in humans, we do know that there is an age-related loss of synapses in humans based on data from temporal bones, that's worked from Charlie Liberman's lab, there is also evoke potential data that are highly consistent with an age-related afferent pathology, meaning smaller Wave I amplitudes in human research participants, even in the presence of normal threshold sensitivity, so we have both the direct observation of the synaptopathy pathology, as well as the indirect evidence of the evoked potentials in the living human patients from whom we can't get the synaptic pathology direct observations.

So there's really significant questions for humans about where risk begins and how risk grows, and one of the things that is really unclear is the repetition of noise exposure, and the repetition of small temporary threshold shifts, I mentioned in the previous slide that you need a much higher level, longer exposure in non-human primates than in rodent models, but if you think about many of the humans who are exposed to noise, occupational noise exposure is a major issue, and you have workers who are exposed day after day, and not potentially to exposures to noises that may not cause a synaptopathy with a single exposure, as has been induced in the animal models, but we simply don't know what the risk is for synaptopathy with those repeated exposures day in and day out, five days a week over a 40-year working career.

So there's really important questions in humans about vulnerability to noise-induced synapse loss, where it begins, how it grows, and what are the differences between

chronic noise exposure and acute noise exposure, in terms of the risk to humans. The other thing that is really important as we think about trying to identify patterns of pathology in humans, patterns of deficits in humans is the issue of individual variability. We know that there is a huge range of variability in the amount of hearing loss that's observed across individuals, both for aging and for noise exposure. If you've ever looked at the ISO tables, there can be as much as a 40 dB difference in vulnerability between the 10% most vulnerable, the 90th percentile, the least vulnerable.

So we know that there are huge differences across individuals in their vulnerability, and there is a lot of work that's being done to try and understand what causes those individual differences. And some of the variability is likely due to genetics, and in particular, some of the genes that are associated with endogenous antioxidants have been shown to be associated with vulnerability to noise injury. Cardiovascular health, cardiovascular disease, including things like diabetes, are associated with differences in vulnerability, the amount of amplification that occurs in the ear canal and the outer ear differs from individual to individual, there can be as much as 20 dB difference in how much amplification occurs at four Kilohertz across adult ears, the ears that have greater amplification may be delivering more power to the middle ear, and therefore putting an individual at greater risk.

There's differences in the transfer of energy through the middle ear. So some ears are more efficient at transmitting energy through the middle ear, Ah, there's been work on dietary nutrient intake and the antioxidant content in diets, there is work showing that circadian rhythm matters if a mouse is exposed in the light cycle, as opposed to the dark cycle, there are differences in the amount of hearing loss, noise-induced hearing loss that's observed, hormones influence noise-induced hearing loss, there are gender differences, there are race and ethnicity differences, so there's many, many factors that contribute to differences in individual vulnerability in threshold shift, and it would not be

unreasonable to predict that many of these factors which influence threshold loss may also influence vulnerability to synapse loss.

But we simply don't have any data from animal models or from humans on what are the factors that might influence degree of risk for cochlear synaptopathic loss. One of the huge questions is how much exposure is safe for everyone, and from a threshold perspective, there are two key reviews that I wanna point you to, these both come from Rick Neitzel, one with Brian Fligor, and one with Ben Roberts, with reviews of noise injury in adults and children. And in both of these reviews, one focused on adults, one focused on children, they found that in order to protect everyone, if we wanted to eliminate 100% of noise injury in even the most vulnerable individuals, then 75 dB-A sound exposure limit would be necessary to completely eliminate the risk of threshold shift, of permanent threshold shift from noise exposure.

Little bit less conservative sound limit of ADDBAL LEX, the eight hour equivalent level would protect all but the most vulnerable individuals against noise injury. About 2% of the population would be at risk for noise-induced hearing loss with the ADDBA, eight-hour threshold shift. But what these data, what these conclusions don't tell us is if these exposure limits would also prevent any risk of cochlear synaptopathy, if they would also prevent any risk of noise-induced tinnitus, if they would also prevent any risk of noise-induced hearing and noise deficits, because we don't know where risk begins for tinnitus, and for hearing and noise deficits, or for cochlear synaptopathy, as we've been discussing. And we don't know how risk grows with increasing sound exposure level, or increasing duration of the sound exposure.

So we don't have good ways to identify who the most individuals are in advance of noise injury, because of all of these different individual factors, so ideally, we would be able to reduce sound levels to protect everyone, but that's really challenging in occupational settings, so these are really important challenges, these are really tough

problems to solve for how we might ultimately prevent noise-induced hearing loss in a larger subset of the population, and prevent other noise-induced hearing disorders in a larger subset of the population, earplugs have been the default solution, but clearly earplugs are not providing a very effective solution, because they're not always worn correctly, they're not always worn consistently, they're not always available when somebody needs them, so despite the requirements for use of hearing protection, we continue to see noise-induced hearing loss as one of the top two occupational injuries, we continue to see auditory symptoms, including tinnitus and hearing loss, as some of the top disabilities for veterans in the armed services.

Some of the data that we collected a few years back, this is data that Sarah Grinn collected in my laboratory trying to understand where hearing and noise deficits began, there was also extensive data collected on otoacoustic emissions, and evoke potential amplitudes, in this study, audiograms were also collected, and these were 28 participants who attended recreational events that they considered to be loud. They were not told where they needed to go, or how long they needed to stay, or how long it needed to be, so there was a wide range of levels. Some of the events were as quiet as 73 dB the loudest events were 104 dB, the average levels that recorded at the events were about 93 dB. The average duration that they stayed was four hours, the shortest duration was one and a half hours, the longest duration was 16 hours, those two points at the extreme ends, the 108 to 110 dB time-weighted averages, those are individuals who went to a music festival, so those were the 103-104 dB kind of range for up to 16 hours at these long festivals.

So what the participants did is they attended an event, they had audiograms and evoke potentials assessed prior to the event, and then they came back the day after the event to parallel the mouse work in which TTS is measured the day after the noise exposure. And at that point, all of the audiometric changes had recovered, there were no changes in Wave I amplitude, there were some borderline significant decreases in DPOAE

amplitude potentially, consistent with a recovering outer hair cell injury, temporary outer hair cell injury, and the one finding that was the most striking to us was, changes on the word and noise test, the words and noise test that was developed by Wilson at the VA, by Rick Wilson at the VA.

And the way that I've plotted the data here is the time-weighted average, so this is the eight-hour equivalent sound level, the continuous sound level that would have been presented in order to deliver the amount of energy that was actually incurred by each of the participants, based on their individual exposure level, and exposure duration. And the red lines here show you the OSHA 85 dBA action level, and the OSHA 90 dBA permissible exposure limit. So below the 85 dBA action level, you see very, very little evidence of changes in word and noise performance the day after the exposures in any of the participants, and above the 90 dBA time-weighted average. You see that essentially every participant had some amount of change, some amount of negative change on the word and noise test, and between those two levels between 85 and 90, it's a complete mix in terms of whether participants had deficits or not.

And so there was a statistically significant relationship, the linear regression line is shown for you in red there, where the more noise there was, the greater the deficits were, and the observation of deficits seems to line up very well with these artificial boundaries, the 85 and the 90 dBA boundaries that have been set by OSHA. When the participants came back a week later, everything had fully recovered, in all of the participants, there were no audiometric changes, and no word and noise changes, and just to be sure that this is really clear, at that 24-hour-test time, there was no TTS, the participants did not have threshold shift at the time, the word and noise measurements were observed, so word and noise testing appeared to be more sensitive than the audiogram at that 24-hour post-noise test time.

This is just looking at the individual SNR levels that are tested within the word and noise test, so if you're not familiar with the words and noise test, the SNRs range from zero dB, where the words and the background babble are at the same level to 24 dB SNR, where the words are 24 dB louder than the background babble. And you can see that at the better SNRs, from 12 to 24 dB SNR, you really have no deficits in any of the participants. What was interesting is the poorer the SNR, the more compromised you see on the task, particularly in that group that had the highest doses of noise exposure. So you can see it eight dB SNR, at four dB SNR, there's some pretty big drops.

At zero dB SNR, you don't see as much of a change, because the participants are already generally doing pretty poorly at zero dB SNR. So I mentioned occupational noise is very different from most of the work that has been done with cochlear synaptophathy, because individuals are exposed for multiple hours per day, day in and day out. And so we did a systematic review looking for ABR data from noise-exposed workers from workers who were exposed to occupational noise, and found 13 studies that included ABR data from populations with occupational noise exposure. And absolutely, there were multiple reports, of the papers that reported latency, Wave I, three, and five latency was typically delayed, there were a smaller subset of those 13 studies that looked at amplitude of Wave I, three, or five, but when amplitude was reported, it was generally smaller, and so the very high prevalence of ABR waveform deficits across these occupational noise exposure groups really contrasts with the inconsistent deficits in other studies looking at leisure noise-exposed groups.

However, the challenge is that in all of these studies, the workers also had significant permanent threshold shift, and so these are not hidden hearing losses, their overt hearing losses that are accompanied by decreases in Wave I, and three, and five amplitude. So there may be a dual pathology to both the outer hair cells, and the auditory nerve fibers, there's really no way of determining what the cause of the deficits was in the studies from noise-exposed workers. But I do wanna make the point about

occupational workers, and some of these hearing complaints, Connor Janssen and Aaron Cochran are PhD students in my lab, and Connor collected a very large survey set of data, this was during COVID, when research largely had to move virtual, and so this is a survey study and online survey study, and it recruited workers who are required to wear hearing protection devices, supervisors of workers who are required to wear hearing protection devices, and hearing conservation program managers.

And 60 to 65% of the individuals who participated in this study reported at least one hearing complaint, and you can see the rates are pretty similar across those three groups of participants, and what I would like to point your attention to is that 15 to 20% of each of these groups reported a diagnosed hearing loss, whereas more than 30%, 30 to 35% reported tinnitus, 35 to 40% reported hearing and noise issues. So tinnitus and hearing and noise difficulties were twice as common as diagnosed hearing loss, and again, neither tinnitus nor hearing and noise are monitored in occupational hearing conservation programs, and we don't have damage-risk criteria for these noise-induced hearing disorders. So these are some real challenges for monitoring early complaints on the progression of complaints in workers.

And some of the data that were, you know, particularly interesting to us from this study were related to audibility needs, 67% of the employee participants reported that they sometimes remove their HPDs to hear better while they're working, when they need to hear equipment, or talk to coworkers and so on. And the supervisors and hearing conservation program managers had very good understanding that employees engage in these behaviors of removing their HPDs in order to hear better while they're working. I mentioned some of the reasons for removing or less securely fitting their HPDs, communicating with coworkers was a big one, 75 to 80% of the participant groups reported this "Can't hear important sounds when wearing HPDs" was frequently reported, to hear machinery or processes better, 25% reported this.

There were some other reasons that were not audibility-related, discomfort was one, and not working well, HPD's not working well with other safety equipment was another. So I hope that I've convinced you that superthreshold deficits are a major clinical concern regardless of whether the synapse loss or outer hair cell loss is the cause, if you're not familiar with the HLAA Voice of the Patient report, the Hearing Loss Association of America Voice of the Patient report, 92% of responses included difficulty hearing a noise is a health concern. 82% of the responses included difficulty hearing a noise is one of the top three most troublesome health concerns. Looking at data from clinics, about 10% of patients seen for hearing and noise complaints have no audiometric loss at the time of testing, so this is a real challenge for patients.

And with that, I'd like to shift gears a little bit, and talk about what data are being collected in clinical trials, given that we have these superthreshold hearing problems that are very important to patients, but which are less well-understood. So as we talk about clinical trials, I wanted to briefly introduce you to the phases of a clinical trial, the first phase is the first in human study, it's the first time a new drug is being administered to humans, it's typically healthy volunteers, and it includes a lot of safety measures, to make sure that kidneys aren't being affected, liver's not being impacted, that you don't have side effects, profile adverse side effects, you know, headaches, nausea, et cetera, that would outweigh the potential benefits of the drug.

These are typically small studies, maybe 20 to 30 participants. In phase two clinical trials, this is when you initially begin to study the efficacy of a new drug in the affected participant population, so assuming the drug was safe in phase one, didn't have major contraindications in phase one, it'll move to efficacy testing, there's a still a continued evaluation of safety, and these typically have somewhat moderate sample sizes, maybe about 100, but that's going to depend on the desired statistical power, and other statistical considerations, such as the predicted mean differences, the targeted mean differences, and the expected variability of the response. Phase three are your large

studies, they're very often multi-site studies, they're used to determine benefits relative to placebo control, if there's no standard of care, or the standard of care, if there is something.

So for example, studies evaluating steroids, or drugs for sudden hearing loss, you know, steroids often serve as the control since patients wanna make sure they're getting at least the minimum standard of care. And these are typically very large studies with several hundred to several thousand participants, so they're large, long, and expensive trials, so you want positive data from your phase two studies in order to be convinced that phase three is worthwhile. As we talk about clinical trials, I want to mention the difference between outcomes and endpoints, so outcomes are the measured variable, for example, audiometric threshold or DPOAE amplitude, whereas the endpoint is specifically what you are looking for within that outcome measure to decide if the drug worked.

So this could be a change from baseline, it could be a reduction, you know, in threshold shift at four Kilohertz, a reduction of at least 10 dB, or the average reduction. This is your most important income, and it tells you whether the drug was successful in preventing disease, or was better at preventing disease than the standard therapy. This is how you communicate with the FDA about whether the drug was effective or not, did it meet the criteria that you set up as the level of benefit that would be required to show a clinically significant improvement. Secondary endpoints can be mechanistic, a drug for osteoporosis could have fractures as a primary endpoint, and improve bone density as a secondary endpoint, the patient doesn't notice bone density, but bone density as a mechanistic insight, you could similarly think about outer hair cell survival, where you might have, you know, audiometric threshold shift as your primary endpoint, and improved OAE amplitude as a secondary endpoint.

And you can have situations, where there's multiple endpoints, if you don't have consensus about which one will best serve the study goals, or if in effect on any one endpoint would be sufficient evidence of effectiveness. So we're starting to see, particularly in gene therapy trials with two primary endpoints, one of which is threshold shift, and one of which is hearing and noise. Then the indication is the particular disease. And so your endpoints and your outcomes would be selected in order to support an indication. So if we think about cochlear synaptopathy, or hidden hearing loss, there's challenges in matching endpoints to indications. The changes in the ABR amplitude or other evoke potentials that are, you know, really serving as a primary outcomes of interest in most of the basic research that's being done are not something that would be by themself notable to the patient.

These would be appropriate as secondary endpoints, not as primary endpoints. So we really have to be thinking about hearing a noise, tinnitus, hyperacusis as the primary endpoint if we're thinking about cochlear synaptopathy, and evaluating drugs that are intended to repair synapses. So ultimately, it's up to the FDA to decide what constitutes clinically significant benefit, but as companies are thinking about their clinical plans, the clinical development, these are things that they're very interested in, and if you are interested in being able to point patients to clinical trials, having data on their baselines for these kinds of measures, you know, will be extremely helpful to determine if they're potentially eligible to participate in a study, so that your data match what's being evaluated.

Now there's many possible outcome measures for which endpoints could be derived, the audiogram, word recognition, hearing and noise, tinnitus surveys are three that I will focus on, simply because those are the most likely to be primary endpoints that in tympanometry otocoustic emissions electrocochleography are more appropriate as secondary endpoints, and we have done a review of the clinical trials that are posted on clinicaltrials.gov. All of the clinical trials that are funded by MIH, and all of the clinical

trials that are reviewed by the FDA are required to be posted on clinicaltrials.gov, but there are other trials posted there, as well, not everything that's posted on clinicaltrials.gov is necessarily funded by NIH, or something that is overseen by the FDA.

In this review, there were nine clinical trials evaluating noise injury otoprotection, 30 evaluating drug induced hearing loss otoprotection, nine evaluating therapeutics for acute sudden sensorineural hearing loss, so hearing loss that has developed suddenly within the previous, typically 48 to 72 hours, and then there were 11 clinical trials evaluating hearing benefits in patients who had stable sensorineural hearing loss, including age-related hearing loss. Now, of these trials, some of them get terminated early, some of them are still in progress, some trials are not going to succeed in meeting the study endpoints, but I wanted to give you some sense of the research that is planned, or in progress, that's posted on clinicaltrials.gov. You can see that for noise injury, most of the drugs are oral, as you look at drug-induced hearing loss and sensory neural hearing loss, there are also intratympanic and intravenous indications, this makes sense that you would see more intravenous delivery for drug-induced hearing loss, because if you think about immuno-glycoside antibiotics or cisplatin, they're very often being given in hospital settings as infusions, and so delivering a protective agent is not necessarily a problem since they already have an IV set up.

These are some of the drugs that have been administered, you can see that some drugs are given in multiple ways, dexamethasone administered both orally and intra-tympanic, methyl prednisolone, N-acetylcysteine, as well. The audiogram is by far the most common endpoint measure in hearing loss prevention trials, you can see that almost 100% of noise injury trials use threshold shift, drug injury is not necessarily threshold shift, a lot of the ototoxicity criteria, in particular, the ASHA significant ototoxic change criteria are used, this would be what percent of participants have an ASHA-significant ototoxic change, and you would look at the percent in the placebo

group versus the percent in the treated group. The other endpoint measures, we've been talking a lot about hearing and noise changes in tinnitus, you can see that these are very infrequently included in most of the clinical trials that are planned, or in progress, hearing a noise in very few trials, tinnitus changes are in about half of noise injury trials, given the extent to which tinnitus is a comorbidity of noise-induced hearing loss, it's a surprisingly low rate at which tinnitus measures are included.

If you look just within the noise injury literature, this is not just clinicaltrials.gov, but also the scientific literature at published clinical trials, you can see that in some cases, drugs are given prior to noise exposure, in some cases, they're given after noise exposure, there are a handful of studies that have looked at prevention of permanent threshold shift, but the vast majority are looking at prevention of temporary threshold shift, again, average threshold shift is the predominant measure. You can see DPOEs have been included in a small handful of these clinical trials. If you look at endocochlear, CAP compound action potential electrocochleography, ABR included in a very small number of trials, changes in hearing and noise are also included in a very small number of these trials.

So we're really not looking at some of the measures that are important to patients. And I just would like to comment that tinnitus trials are really not standardized either. There's a review of 66 clinical trials by Jen and Tyler that revealed a wide assortment, 37 different scaling, 37 studies used scaling methods, questionnaires were also commonly used, you can see some of the most common ones listed here, patient self-report was used, there were some psychophysical techniques that were pretty rarely used. So really very little standardization of these measures in clinical trials. Most of the efforts to try and study prevention of hearing loss are focused on antioxidants, glucocorticoids, or JNK receptors, this image is from an excellent review from Vanderwaters Lab by Denital, highly encourage it if you're interested in how different drugs work to protect different cells in the inner ear, gene therapy is really different,

gene therapy is intended to drive the development of new cells to reinitiate developmental cascades, as opposed to preventing cell death.

And there is a significant amount of commercial activity, this is a review from Ann Schalder's group in 2019, where they found 43 different companies with preclinical or clinical research programs. And this is a phenomenal change from the early 2000s, when there were, you know, a very small handful of small startup companies, now you have major pharmaceutical companies, large venture capital, large biologic companies, really an incredible amount of activity trying to develop drugs for hearing loss indications. And if you're not already aware, I did wanna share with you that the first drug for a hearing loss prevention indication has been approved, this is PEDMARK in the United States, it was approved in September of 2022, and it was more recently approved by the European Commission for use in European member states, and this is a sodium diose sulfate formulation that is approved for use in pediatric patients one month and older, with localized non-metastatic solid tumors.

So a drug that reduces the cisplatin-induced hearing loss in pediatric patients who are treated with this. The last thing I wanted to share with you was just related to investigational therapies for cochlear synaptopathy, which we've already touched on, neurotrophic factors are of really high interest for synaptic regeneration, there are animal data that suggest restored synapses and restored Wave I amplitude, but again, going back to our primary endpoint questions, the clinical correlate, the functional measures are really critical to understand and identify for clinical triads. So what guidance can you take with you today in terms of information for patients, at this time, there's no drugs that are approved for prevention of noise-induced hearing loss, and really the level of evidence for dietary supplement benefits is very low, even though antioxidants are one of the primary categories of interest.

But noise injury is preventable with known tools, and this is true for both permanent threshold shift, and it'll be true for cochlear synaptopathy, as well, to the extent that it's induced by noise and not age. Decreasing the exposure level and duration are by far the most effective, hearing protection devices are effective, when they're used consistently and correctly, and please remember that there's diverse options that you can recommend for your patients. There's hearing protection targeted for musicians, there's electronic HPDs, which are fantastic for hunting and target shooting, so really let your patients know there's some great options for hearing protection and preserving their hearing. These are the references I have included in today's presentation, these are the PhD students who I mentioned collecting data on these projects, and I'm happy to take any questions or discussion points that you have related to what we've talked about today.

Thank you so much for your interest. I know I've thrown a lot of information at you. Well, if we don't have questions, I will thank you for your time, thank you for your attention, my contact information is here, and I'm happy to field questions after the webinar. Have a great rest of your day.