**HHF Research Webinar Transcript**
**Accessing the Inner Ear for Treatments**
**Monday, April 3, 2023 | 5pm ET**
**Tony Ricci, Ph.D.**

CHRISTOPHER GEISSLER - Welcome to our Hearing Health Foundation research webinar. I'm Christopher Geissler of HHF, and I appreciate you joining us today. Dr. Anil Lalwani, our usual host whom many of you are expecting, will be joining us for the second half of this webinar during the Q and A session.

Today's topic is accessing the inner ear for treatments. How do we deliver drugs to the inner ear, which contain the hardest bones in the body, and is a closed mechanically sensitive system under pressure? How can we inject medication into this system to deliver drugs without disrupting the system itself?

This event has a live captioner and is being recorded. You can enable closed captions by clicking the CC button in the toolbar that's at the bottom of your screen. If you need any other assistance using Zoom, follow the link to the technical guide shared in the chat, which is also at the bottom of your screen.

I am the director of program and research support here at Hearing Health Foundation, where I help administer the Emerging Research Grants program, affectionately known as ERG. ERG is a competitive program that awards funds to researchers conducting cutting edge hearing and balance research.

Our presenter today is Tony Ricci, PhD, who will discuss recent bindings from his lab that solved the issue of accessing the inner ear for drug delivery without adverse effects. A 1999 to 2000 ERG awardee, Ricci is the Edward C. and Amy H. Sewall professor at Stanford University School of Medicine, and a professor of otolaryngology-head and neck surgery, and of molecular and cellular physiology. His work has focused on hair cell function, using electrophysiological and imaging tools for his work. He also has a translational component to his work where he is collaborating to develop non-ototoxic antibiotics, developing new drug delivery systems for the ear to facilitate gene therapy treatments, and more recently investigating how hearing loss impacts cognitive function.

The ERG program that provides seed money to scientists just starting out in their field of research is only possible through the generosity of supporters like you. If you'd like to support our work on hearing loss, tinnitus, and related conditions, you can do so today or at any time at hhf.org/donate. And now we'll move on to Dr. Ricci's presentation. Please remember to ask your questions through the Q and A box also at the bottom of your screen, and Drs. Ricci and Lalwani will try to answer as many of them as possible following the presentation. Dr. Ricci.

TONY RICCI - Okay. Okay. Thank you. Thank you to the foundation for inviting me today, and thank you all for coming to listen. So I'm going to try and tell a story that's about--it takes about 20 years to accomplish. But I think it is meant to give you an idea of how basic science research leads to translational findings. And being a really, a basic scientist in a clinical department, to me, most of the work that I do is in fact geared toward having applications. So understanding the underlying principles of how hearing works, for example, means that we'll have a better understanding of how dysfunction works and how we can intervene. And so the story I'm going to tell today is one

example, I guess in my career, of how this all happened. And it really started with the grant that I received from the Hearing Health Foundation. So I think it's appropriate to start there at the beginning.

So a little background. Hair cells are the sensory cells of the inner ear. They get their name because they have the sensory organelle called the hair bundle. And when this hair bundle is deflected by vibration and sound, it causes force on the bottom end of this link here, and that opens ion channels and creates an electrical signal. And so to zoom in on this little area, you'll see that there are ion channels down here that are connected to this link. So when the bundle moves, the mechanical force activates these channels.

I've spent a lot of years studying the properties of mechanical transduction, and this ion channel. And when I first started my independent career back in '99, one of the first projects we were--I was interested in doing, was really characterizing the physical properties of this channel. At that point, we didn't know the molecular underpinnings of the channel.

I was interested in figuring out, for example, like are there binding sites in the channel, and where might they be, and how big is the channel, how wide is it? What can fit through it? And the Hearing Health Foundation actually gave me money to start this investigation. And so we did a lot of work and we found largely that this is a very big pore. Much bigger than what people had originally thought, and so lots of things can go through it, and this is just showing different ions that can get through it and that there are binding sites. But we found in fact that it was so big that large molecules can get through it, but not only that--that the channel is rectified. And what that means is that things can go in through the channel much better than they can come out. And so ions will go in at a high rate, but they'll come out at a much slower rate.

This got us thinking about aminoglycosides. Aminoglycosides are an antibiotic, the most widely used antibiotic in the world. They're used a lot here, for example, on infants in the neonatal intensive care, and they're also used in cystic fibrosis patients a lot. And there's a big side effect, which is deafness. And the aminoglycoside selectively kill the sensory cells. Oh, sorry. So this is an aminoglycoside, and what we found is that it can go through the ion channel. And so the question is, well, if we stop it from going through the channel, will that stop toxicity?

The first test was we made Texas Red Gentamicin. So we made an aminoglycoside that we could see. We weren't the first to do this, we got the recipe from Peter Steyger. And what we found was, this is now a cochlear explant and you can see the red stripe, and the red stripe are the hair cells. So the hair cells all take up this gentamicin. And so we said, okay, if we block our favorite ion channel, in this case with curare, you can see that the drug doesn't go in. And so to us this suggested that, well, this is why hair cells are selective, because they got this big channel that the drug can go through. So if we can stop it from going through this channel, then we would stop the ototoxicity. Now the caveat here, and we'll talk about this more later, is that this is neonatal tissue and it's an explant. And so access to the drug is different than in vivo.

So, the question is, if we don't let the drug in, can we make it not--will it then not hurt the hair cells? And so to do this, and I know this looks complicated, but don't worry about it. This is simply the crystal structure of of the ribosome that the aminoglycoside binds to. So when it's killing bugs, it binds to this ribosome. And so what we want to do is make it still kill the bugs and not kill the hair cells. And so we looked at where does it bind in the ribosome, and we selected targets where there was space to put another molecule on that wouldn't interfere with binding. So again, this is the

aminoglycoside, this is the channel. The idea is we're going to stick something on here that stops it from going in the channel, but all of this is still available to interact with the ribosome. So that was the idea.

We made that compound. And this is the result. So this is the histology where you can see three rows with outer the hair cells, and a row of inner hair cells. The parent aminoglycoside kills all of the hair cells. So there are no hair cells here. And the new compound, you keep all of the hair cells, so the hair cells don't die. So this supports the idea then, that if we don't let the drug in, it can't kill the hair cell. Again, this is neonatal tissue.

We tested it in a model of deafness in adult mice. So this is called a auditory brainstem response. So what we're measuring is the electrical activity of the brain across at different frequencies of sound, and different loudness of sound. So we increase the intensity. So the better you hear, the lower this number is, because it means the softer the sound you can hear. So this is control. And when you put a typical aminoglycoside on, you can see that now these animals are deaf. When you put our new compound on, you can see that the animals are no longer deaf, that they can tolerate this. And there's a maybe a little bit of loss at high frequencies, but in general, it's much better than the parent compound. So this is exciting to us. But again, this work is in neonatal cultures, which is not their normal environment. And here we're not measuring directly uptake into the hair cell, we're measuring the fact that hair cells are dead. So it's a correlation and not causation.

So there's been bickering around, well, is what's happening, what you really think is happening? So legitimate questions, the problem is then how do you answer that question? And so what we decided was we need to measure uptake in the cochlea in the adult while it's happening, so we can see directly what's going on. So you might say, well, why has--this would be a good thing to have, right? There's lots of experiments you could do it, potentially could be diagnostic, why hasn't this been done before? And the - largely the reason is because it's really hard to do. And if you look, the cochlea, which is a bony structure is here, and this is the whole inner ear. You have facing outward, the middle ear. And if you do any damage to that, you're going to lose hearing. Surrounding the inner ear is all bone. So it's all bony structure. And if it's not bone, it's blood. So it's a really difficult place to get to. And if it's not bone or blood, it's brain.

So it's really hard to get at the cochlea. But even if you get to it, there is still two other problems. The cochlea itself, now we're just looking through a cross section of it, is surrounded by very hard bone. And inside the cochlea, it's basically this coil structure, and there are three fluid-filled compartments. And there's a pressure gradient in the cochlea and across these compartments, because if you zoom in on this spot, the sensory cells are here and it's this vibration of what's called a basal membrane that's stimulating these cells, and that's driven by this pressure difference. So if you do anything that hurts the pressure, you're going to lose hearing. And these mechanical receptors are some of the most mechanically sensitive cells in the body. So getting through all of this bone without mechanically stimulating them is also not a trivial thing to do. We decided we were going to try to figure this out.

The first step was, well, what was the view that we want? And so we decided, because we're working in mouse, it was actually somewhat limited. We're going through the apical turn, and we're going to try to come through, kind of through the stria so that we get a look at the hair cells, and we should also have access to the spiral ganglia and the nerve fibers that way. So that's how we're going. So this is a cartoon of a mouse just to show you how we're going in. So the cochlea is in

blue, the bulla which is the bone around the cochlea is in this translucent light. So first we have to go through the bulla, then we have to make this, we're calling it an imaging window on the cochlea.

Now the TM here is the tympanic membrane. So we need, there's a very small region that we can get at, and that's where we're going. So how do we get at the two issues, the mechanical sensitivity and the pressure? So in the first one is the mechanical. So what we decided to do is we developed a, what we're calling a chemical mechanical approach. So we use this phosphoric acid gel to dissolve the bone, and then we scrape it away, and then we dissolve the bone some more. So it's a very gentle approach. This phosphoric acid gel is actually what dentists use. So those of you who have ever had a cap put on your tooth have had this done. So after they clean up the bone, before they put the glue on, they put this gel on, it punches holes in the bone, then they put the glue on, and that makes it stick really hard to the cap, so the cap doesn't pop off. I spend more time in the dentist's office than I like, so that's where I learned about this stuff.

We tried--so that's the approach. And then the second approach is, so there's this thin membrane here called the endosteum. And it basically separates between the bone and the intra-cochlear fluid. And so our thought was, if we can get the bone off without damaging the endosteum, we would keep the pressure intact, that was the idea. And I can tell you I've done thousands of dissections. I'd never heard of an endosteum and I'd never seen it, because every time I take the bone off the endosteum goes with it. And so we found it was there. And so our approach is to thin the bone, and then just to pick it off to keep the endosteum in place. So just to give you an idea, we put the gel on, it punches holes in, then we scrape that down, and then we do it again, and we scrape it till it's really thin. And then we can come in with a needle and just peel this little bit of bone and leave the endosteum intact. So that's how we do it. Just to show you, this is the eardrum, the tympanic membrane. This is the bulla that we want to open. And you can see there's a big old blood vessel right here. So we don't have a whole lot of space. So we put on the gel, and then when we peel it away, you can see that we leave the tympanic membrane is fine. You can see the blood vessel here. and now you can see the cochlea is exposed right here. So then we go to the cochlea and we use the gel again, we put some gel on the cochlea, we thin that down, and we can make this imaging window.

You can see the window here. You can also see there's no fluid coming coming out. And that's really the tell. If there's fluid coming out then we took the endosteum away. So this again is the hearing test where lower is better, and this is across frequency. And what you can see is that each stage of the surgery, we have no hearing loss. In guinea pig, we've actually--it's a recovery surgery where we've gone for weeks and the animals are fine. In mice, we've gone out to about five hours. And this is about what you can see. So you can see three rows of outers, and this is the tunnel, and this is the inner hair cells, and this is where the nerve fibers would be. So, this is really the first kind of live, functioning cochlea at the cellular level imaging that's been done.

So back to the question, do aminoglycosides go into hair cells through this channel in the adult cochlea? So now again, this is the view that we're going to have. We're using the same drug, the Texas Red-labeled gentamicin. And here we're looking at the stria, and you can see the blood vessels in the stria where we put it in IV, it goes to the stria, and then over time you can see it's reducing. And it's reducing because it's being cleared out of the bloodstream. So then we look deeper at the hair cells, and you can see even at 10 minutes you can start to see the hair cell signal, and that signal is growing. And now you can see three rows of outers and a row of inners pretty clearly. And there's really no other cells that are accumulating the aminoglycoside.

We can see it now for the first time ever, in real time, hair cells taking up the aminoglycoside. Is it going through our favorite channel? So to test this, we used an animal, a genetically mutated animal where they do not have the mechanotransducer current. So in that case, again, when we look at this stria, it looks pretty comparable. It fills up the blood supply, and then you can see it's slowly reduced over time. When you look however at the hair cells, there's no accumulation in the hair cells. So this suggests that in fact, the answer is yes, in vivo, in adult mice, the aminoglycosides are going through the mechanotransducer channel. So there are a lot of experiments that we can do now with this preparation. And part of the math, I guess, that I do when I'm deciding what to pursue is we developed, I mean, developing that technology took almost four years to do. And honestly, it was not supported by NIH, because NIH kept saying, well, you can't do it, it's not possible to do it, so we're not going to pay you to do it. So it's been foundations and philanthropic dollars that supported making this happen.

Now that we have it, there are a lot of basic science experiments we can do. One thing that would be really useful is can we apply locally drugs into the inner ear now, and study their responses in the hearing organ for basic research purposes. But also, again, it's one of those cases where a local delivery of drugs into the cochlea would be really translationally useful. If we could put protective drugs only into the inner ear, say, during cisplatin treatment or something like that, so cisplatin gets the cancer and the ear still keeps working because it's protected, that would be a simple example. And so we decided this is something we wanted to look at. So we went at drug delivery through the semicircular canal.

Now again, we were not the first people to inject a drug through the semicircular canal. And I think what we're bringing to the work: one, is an optimized approach- surgical approach-to make things very reproducible and qualifiable, and not damaging in and of itself to either the ear or the vestibular system. And the second thing is characterizing specifically where the drug is, where the drugs are going. Is it perilymph, is it endolymph? Is it both, is it reproducing them? And so I want to show you a little bit about that. So this is the human ear. You can see where the semicircular canals are. In the mouse, we're going to go back here to the semicircular canal.

We first tried this, we did this experiment before we had a good surgical approach for it, and we simply compared round window injection with a dye to semicircular canal injection. And this is the methylene blue. And you can see with a round window injection that it doesn't get all the way to the apex. Yet when you do a posterior semicircular canal, you can see it's really pretty unfilled, it's pretty uniform. And we did this in adult as well, and round window injection in adult was even worse than with neonatal, and that it was not getting to the apex. And yet when we do posterior canal, you can see that it fills nicely.

Now there were two issues here. First, with these experiments, we couldn't differentiate which compartment we were actually hitting, perilymph or endolymph. And secondly, with our initial experiments, there was some hearing loss associated with it. So we decided to modify how we do the cochleostomy to the canal injection. So here's the set, we isolate the semicircular canal, just showing you here. We used this blue gel and we dissolved the bone. So you can see that here. So there's just a little hole, and we have really good, the bone doesn't crack, we can make it exactly the same size, it's very, very reproducible. And then we can put our tube in the hole for drug delivery. And then when we're done, we tie the tube off, we actually leave the tube in the ear, or in the semicircular canal. And just to show you the CT scan. So here's the canal in the--this is the mouse. And now we're going to flip it over so that you can see the canal better where we do the

canalostomy here and not here. If you zoom in, you can see where the canalostomy is for. And you can kind of, if you use your imagination, see the tube. And then this is without-the control side.

Are there functional effects? So we did what's called VSEP, which is basically a vestibular evoked potential. And here I'm just showing you an example. So again, you basically, you're vibrating the head while you're measuring electrical output. This is going up in intensity, so the red line is what we're calling our threshold. After injecting artificial perilymph, you can see that there's basically no effect on the threshold. And then similarly, we did the auditory testing. Here I'm just showing you the one time point, the 16 kilohertz and the raw data. This is what we call threshold. This is getting louder. This is the electrical response. You can come down, see this is where we're first detecting it. An hour after injecting artificial perilymph, basically there's no effect on the hearing. So, we have the method worked, the surgical approach worked. So the question is, where does it go? So we're injecting in the posterior canal, and so it could push down on the membranous labyrinth and only go in the perilymph. It could penetrate the membranous labyrinth and only go into endolymph. Or it could do some combination of both and be random. So obviously this would be the worst case scenario. Either of those would be useful.

So again, the reason this is important is that when you look in the cross section of the cochlea, there are different cells that are exposed to perilymph compared to endolymph. And so, for example, if you want spiral ganglia cells or neurons, you don't want to put the compound into the endolymph. If you want stria, then you you want to get it into the endolymph And with the hair cell, it's kind of a mix. If you want to get the apical part of the hair cell, then you want endolymph, and if you want the basal lateral part, then you want perilymph. So our first test in figuring this out is to use a drug. We put a drug that blocks our favorite channel up here. So if it's effective, it means it's in the endolymph, and you'll have hearing loss. If it goes in perilymph, then you won't. We also could block the synapse where the hair cells talk to the nerves with a drug. And then if it goes in endolymph nothing would happen. But if it goes in perilymph, it should block it. And we could block action potentials in the nerve using a sodium channel blocker. And in that case, again, if it goes in perilymph it should impact the hearing. And if it goes in endolymph, it should do nothing.

That was our first test, that's just a note. So we did curare which goes and blocks the transduction channel. And you can see from the raw data there's no effect. And in the summary data, there's basically no effect. So this would argue that our infusion is not actually into endolymph. And we used CNQX which blocks these receptors, the synaptic component. And you can see there's a big effect. So now the animal cannot really hear, it's more than a 20 dB effect. So that would argue, okay, the infusion is into perilymph. And then confirmation of this, is blocking the sodium channels, the action potentials with lidocaine, and you can see that had a huge effect. And so the animals are basically deaf with that drug. So pharmacologically it suggests that the drug is going into the perilymph, but seeing is believing. So we used optical coherence tomography. So this is a cool technique that allows you to image through the bone in living in vivo conditions. And so what I'm going to show you is we're imaging this part of the cochlea. I'll show you the movie in a second.

This is what we're looking at. These are perilymph and this is endolymph. And so when you watch them moving, you can see the bright spots where the beads are, and the beads are going into into perilymph. So I'll just show you one more time just so you could see. So this supports the idea that it's going into perilymph, and I'm going to just show you one application. So this is using AAV adenovirus where we expressed GFP or mCherry using a semicircular canal injection. And you can see virtually all the hair cells in the utricle are labeled with the GFP, meaning the virus worked and got into all of these cells. This is the cochlea. We got most of the cochlea in this picture.

There's more base here, but what you can see is all the inners are lit up, and in fact, most of the outers are lit up. And if you look at spiral ganglia, so it's saturated here just so I could show you all the fibers. But pretty much all the fibers get labeled, and this is a pretty picture showing how the fibers are all pretty much filled. The green dots are the synapses on the hair cell here, just so you can put it together. So just to summarize where we're at, I think it's important for people to realize that basic science questions can be foundational and important to solving translationally relevant problems. I showed you that aminoglycoside antibiotics enter hair cells through the mechanotransducer channel. And that blocking this entry is protective.

This is now a big target site for generating new aminoglycosides, or preventing aminoglycoside entry into the endolymph space. We've shown you that it is in fact possible to open and image within the cochlea without causing hearing loss. And I'm really excited because this is really a direction that we're pushing the lab in now, both basic science and translationally.

Finally we ended with showing you the semicircular canal injection is a viable drug delivery to the inner ear, and that it targets the perilymph. And just to quickly--short term we're looking at putting pumps in for slow release of drugs to see how that will work. We're optimizing the imaging approach for repeated measures so that we can study noise, aging and regeneration, things like that. How big an animal will the surgical approach work in? We've not tried, human bone is a heck of a lot thicker than mouse. And so we don't know if any of these will work that way, but we're going to see, we're going to try, and we're thinking maybe some combination would work. And similarly with the canal injection.

Long term we're thinking that we can use optical tools potentially as stimulation through imaging windows where there might be hearing loss. So in a sense you could think of it as a cochlear explant instead of a cochlear implant where you could actually put something around the cochlea that would stimulate. And could we better target small implants to regions if we had better access to different parts of the cochlea? And we're looking at potentially developing some diagnostic tools this way as well.

Finally, I just, I showed you literally 20 years of stuff, so I only have kind of some of the people to thank here, and thanks for listening. And I will again say, a lot of what I presented today wasn't really all funded by by NIH. A lot of it is because developing new technologies and whatnot, a lot of times isn't something, NIH wants to take a chance on. And so using foundation money and using philanthropic money is really critical for that, for those efforts, for really getting new things started. So thank you.

ANIL LALWANI - Well I'm just going to clap out loud, Professor Ricci, that was an amazing, amazing talk. Anil Lalwani, chair of the Council of Scientific Trustees, and it's my pleasure this afternoon to moderate the session of question and answers. And you know you talked about the translatability to humans. As you know, in the past there was an operation called fenestration, where we would thin the bone over the lateral canal and release the otosclerosis. And the other thing where we do something similar is when you have a congenital abnormality of the cochlea and the balance canal, sometimes we fenestrate the balance canals to get into the cochlea. So I think you've hit another approach to how we get materials into the inner ear. Any thoughts to why a round window membrane injection wouldn't get all the way to the cochlea, but a vestibular labyrinth, which is very similar in terms of connection, would? Any thoughts about, any insights you can provide there?

TONY RICCI - So the simple idea, so if you look at when you're injecting in the round window, you need some release valve, kind of. So, and I think there are papers that say, if you put a hole somewhere else, you actually get better penetration that way. So that's one. And when you're going from the canal, you're actually kind of going downstream, and the cochlea is in the middle between where you're injecting and where the exit point would be. I think for me, I would really be interested in revisiting what the cochlear aqueduct is doing. So certainly in mice, it's very clear that when you go into the round window and we see this both in neonatal and adults, that depending on how fast you're injecting, you can send most of what you're injecting through the aqueduct.

To me, although I know everyone seems to think the aqueduct is always closed in adult and it's species dependent, I actually think of it more like a pressure release situation. So I think you could use the round window, but you'd have to be really slow in injection, and not open the cochlear aqueduct. If that's true in humans, I haven't done those anatomy there, so you would know more than I would. But I think a lot of it's done on cadavers, in which case this--there's not going to be pressure. So I think it would be interesting to actually look and see if pressure makes a difference there for the translation side of it.

ANIL LALWANI - So you're thinking that there might be actually some loss of material through the cochlear aqueduct, so you can't go through the rest of the cochlea, because you're losing volume? Is that what you're thinking there?

TONY RICCI - Yeah that's right.

ANIL LALWANI - Now is those--

TONY RICCI - See it in the brain, like if you inject too fast in the mouse, then you'll transfect the brain.

ANIL LALWANI - Yeah, as for the original therapy works for exactly that. Back in the past when people were using a pump, in our own lab, we showed how it could go through the cochlea aqueduct. Now I must admit, when you were talking about whether it was the endolymph or the perilymph, I was really cheering for endolymph honestly. Because there isn't a safe way--I mean if you were able to get the material to endolymph, it'd be like a home run.

TONY RICCI - Yeah. So I can tell you, it's one of those things, I'd have lost my house, because I thought oh it's going in endolymph. If you're going in and you're tearing it open, there's not that much space for this tube to go in. That's why we did so many different experiments, because every one we did, I was like, that can't be right, that can't be right. Because then eventually you have to believe the data. I do however think that we can use it for endolymph. What we're doing now, really, you saw the tube. The tube is almost the same size as the canal, and it's very blunt. I could take, I guarantee that I could take a beveled patch electrode and just put it in and it would go right through the membranous labyrinth into the endolymph. The question though is, how much can you inject in the endolymph without doing any damage, and can you get it where you need it to be? Because then the hole is not so conducive to flow I think. But that's actually on the list to try, because I like home runs.

ANIL LALWANI - Now when I was listening to you talk about the mouse surgery, I don't think people realize how small the mouse cochlea is, and what you just accomplished in what you were doing, because they're a tiny little thing. I mean I think your scale showed one millimeter, and one

millimeter is really tiny. And so I just want to congratulate on your amazing feat in actually making such a small fenestration in being able to use that. Any, let's say, do you think that's translatable into human practices some way or another? What are your thoughts about that? The whole using that little stuff to melt the bone away? Is it fast enough or is it too slow or?

TONY RICCI - So what I think is--yeah, so firstly, so in mouse we put it on it's like for 10 seconds, and it goes in and and you scrape it off. So it's pretty quick. In guinea pig it takes tens of seconds, and repeated measures, treatments. But I think in human, I would guess that probably you need to do some drilling down, and probably that's not going to be too damaging. But when you get close then I think you use the gel, and then you scrape the last bit away. And I think that'll save on the hearing side of it. I mean we found out, again, I don't do--humans aren't typically volunteering for this. So in the mouse, like even going through the bulla, there's a percentage of time where you do a little middle ear damage when you're going through. So we use it there as well. And I mean it's been amazing.

I mean it took--Jinkyung Kim is the postdoc who really pushed this in the mouse. It was Jen Alyono and Eduardo Corrales, were two residents that did it first in the guinea pig. And all of them, what they had in common was resilience because none of--like it seems, oh this is easy, or that's the right way to do it, but we probably tried, I don't know how many different ways to do it, all of which did not work to get it. But now it's really, it's standard practice in the lab. So I think it's--I do hope that it can be useful on the surgery side. I'm hoping we'll get some interest in trying it out. Because I mean I think as you're going to--there are a lot of places where the round window membrane's compromised or changed because of the pathology. And so that's now that could be a problem in getting in the way. So yes, it's more invasive, like to get to the canal to do surgery, but if you can use it to get at a living cochlea, and not do damage, it opens a lot of doors for treatment. You don't have to be completely deaf to get treatment, maybe.

ANIL LALWANI - Yeah, no, I have to tell you, I'm kind of envisioning how I might use this in my surgical practice and opening up the inner ear while you were talking about this material that melts away bone. Now we have a, actually a great question from Ruben [inaudible] who says, "Great talk Dr. Ricci. Which strategy do you think has the most potential in preventing ototoxicity, designing novel aminoglycocides that do not pass through the MET channel, or blocking aminoglycoside passage through a stria vascularis as your lab has shown recently?"

TONY RICCI - So that's a that's a good question, and thanks for looking at the paper. So, scientifically I would say the answer is, using both, a combination of the two. So using aminoglycosides that are inherently less toxic because they don't get in as well to the cell, and coupling that with blocking transport would be the optimal way to do it. I think, because it's like a double protector. I think practically getting a new antibiotic through the FDA, we need younger people because I'll be dead by the time it happens. So I think that will be tougher. But the drugs that are blocking the transporters, I think is already approved. And so I think that can be optimized and used now, and honestly what really should be used is with cisplatin because it's a better blocker of cisplatin transport than it is of aminoglycoside transport. So getting--now the problem with the transport that has, it needs its own, I think it needs to be modified itself, but you need to get it there and has to be there long enough to block the transport. So it's going to be finding this balance between them. So short term I would go for that.

Long term though I think, I mean we worked out the chemistry, I mean it also, it was eight years to figure out how to manipulate aminoglycoside so that you can make the compounds that you want,

and we've got all of that. So I'm hoping that, that will be used. And now again the chemistry is, it's not trivial but it's worked out compared to what it was before. So I mean we have three compounds now that are 40 times less toxic than parent compounds, and we have seven more in the freezer that we're running through the tests that are the second generation of it. So I'm hoping that we'll find a way to get them tested in human as soon as they come out, as soon as we can. That's the part of science I don't really know that much about, so I'm going to need some some help on on that part of it.

ANIL LALWANI - Well I was just thinking you could probably live forever if you just go up the street at UCSF, and get those telomere injections, and this lasts forever. But there's another great question from Wafaa Kaf, and this is a very broad question, Professor Ricci, which is, "What about the use of nanomedicine via nanotechnology delivered into the inner ear?" And I think you can take that question any way you want to and kind of broadly address technology out there, or what are your thoughts?

TONY RICCI - So I think, what I've looked at are--and you can correct me if I'm getting on the wrong topic, but there's been kind of liposomes used for delivery of drugs and there's nanoparticles with slow release that have been used for for drug delivery. And I think they all show some promise. The question is getting them across. Most of the time I see it on round window. So if you can find a way across the round window, I think then that can be useful. If you just have one, have a gel that will stick to the round window so it's there long enough for it to penetrate the round window. The problem, and even I did not show this, because I didn't want to go on and on, but even with a local injection in the canal where the drug ends up in the body, or even within the ear, depends on the chemistry of the compound, because the blood perilymph barrier is selected and grows both ways. For example, when we put the gentamicin Texas Red in the canal, you watch the mouse turn red. So you can see it goes up and down the spinal cord, it goes into the paws, so you can see that it's going right across the blood into the bloodstream and being transported.

Similarly, we've seen similar things with AAVs as well, that we can find expression in places not the brain. So not where, oh it flowed into that place, but places that had to get into the bloodstream somehow. So I think, I don't know if you like solve the problem of the round window diffusion with nanoparticles, will you then create the problem of how do you keep it in the ear once you, once you get across. And I don't know honestly know the answer to that. But I think it's definitely a really good way to go at it, because if you can get around the surgery then you'd be better off.

ANIL LALWANI - Well, you raised some really good questions about safety issues in whatever delivery method you might choose and distributions. So that was very useful, I think it's very useful to our audience to know that every route has this potential benefits as well as drawbacks, depending on what you end up doing. There's another question that's kind of interesting, and I suspect it would be broad interest to our audience, which is, "Does salicylate toxicity, aspirin toxicity occur by a similar mechanism?" Is it the same channel, different channel any, I actually haven't looked it up for a while so I don't know what the answer is. And I'm curious, do you know?

TONY RICCI - Well, so it's interesting the--why cells die with salicylate, I do not understand. I know that salicylate has very little effect on the transduction channel. I haven't published that, but I've tested that. And there's small effects. Now it has big effects on the outer hair cell function, because it interferes with prestin and the motor, it shifts that curve. Why that leads to outer hair cell death, I have no idea, to be honest. And there's lots of things I don't have any idea about, but like, why do mutations in prestin cause outer hair cells to die? I mean you're deaf, but why does that make the

outer hair cell die? So it seems to me the prestin, there's something else going on there, and maybe salicylate is a tool to give us some idea of what that might be. Sorry, that's not a great answer, but.

ANIL LALWANI - That was great. There's a question that I'm going to just twist slightly differently to ask your opinion about something, and somebody's wondering, is our goal to repair the hair cells or to replace them? And I'm curious, based on everything you know, Professor Ricci, which direction do you think will be the first one that we're able to successfully do, repair them or replace them? Or are they both equally likely down the road?

TONY RICCI - Depends how long the road is.

ANIL LALWANI - That's actually, that's part of the question too. So actually if you can sort of give us your insight into where the field is heading, and where you think our opportunities are, and how long do you think it might take?

TONY RICCI - All right, so first I have to say it's a little bit of a philosophical question. So how does science move forward? And I think a lot of people think of science and you have this incremental findings. And I think there is, like, that's the baseline, that it's always kind of moving forward. But the reality is, there are big hits and they're usually accidental. And it means that you're doing, if we're not talking technical techniques, and it means that as long as you're doing really careful, rigorous science that when this accident happens, you see that it's real, and then are you clever to understand it and move it forward. And if you believe that really there's this kind of almost randomness to when the big findings happen, then the way to make it happen faster is to have more people studying it.

One of the big limitations that's happened in the ear, I would say is, that it's such a very small field. There's not--if deafness killed you, it'd be getting a lot more attention because it's its own pandemic in terms of the extent of hearing loss, and how hearing loss is happening earlier, at earlier and earlier ages in people, and that people are living longer and longer just makes it the loss of hearing that much more important. But there's not as big a field study.

But that being said, I think both repair and replacement are real possibilities. And I think that repair is likely less complicated. So it's likely to be the first thing to happen in terms of a biological cure for hearing loss. Now it depends on what was broken and when it was broken. There's lots of different hearing losses. I think genetic repair maybe will be possible, but it's going to depend on how early you have to get in there to do it. I don't know how many people are going to say yes in gene therapy in utero in order to solve a genetic problem. I think age-related hearing loss is something that we'll be able to get at with a repair, reasonably fast. The regeneration or replacement of hair cells or stem--so there's--can you make new hair cells in the existing system? That's one question. And then can you just put in cells like stem cells, and make a whole new epithelium or something like that? I think the stem cell side is going to be the hardest to do. I think the re-engineering, re-genetically engineering is going to be the medium term solution to it. That was also very long winded, but I don't know if that helps.

ANIL LALWANI - You know, that was perfect. You heard it here first folks. You got Professor Ricci's opinion about where we're going, repair possibly before regeneration. There's this certain amount of timeline and the Hearing Health Foundation is so committed to supporting every possible avenue for either restoring hearing loss or preserving hearing. Professor Ricci, thank you

so much for a very engaging, very easy to understand talk of very complex topics. Thank you for your contribution to the field. Also want to thank all the attendees tonight for attending this wonderful webinar. We're so grateful to you, our community, for your support of our emerging research grants program. Remember, that you can donate to our efforts to advance better treatments, those that Professor Ricci was just talking about, and cures for hearing and balance conditions at hhf.org/donate. Thank you and please enjoy the rest of the day. The sunshine's out there.