21st Century Audiology: Hair Cell Regeneration, presented in partnership with Salus University
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- [Moderator] At this time it is my pleasure to introduce Doctor Radhika Aravamudhan who is the Dean of Salus University's Osborne College of Audiology. She is responsible for the development, implementation, assessment and accreditation of the University's on-campus Doctor of Audiology program, as well as the Master of Science in Clinical Audiology program and summer workshops. Thank you so much for being with us today and at this time I hand the mic over to you.

- [Radhika] Hello, everyone, and welcome. We have a quick poll coming up. So if you could please let us know where you're attending from, it really gives us a good idea of who our audience are and where you're coming from. Okay, I see several. California, Colorado, Maryland, Ontario, Canada, Michigan, Seattle. Good, okay, so it's a sort of all around. Okay, so as you're typing those answers, I wanna go ahead and get started. Considering most of you are from the U.S., the things that I'm gonna talk about today are not gonna be very new to you. You probably are already aware of it, but I wanna set the stage just a little bit as to why we thought me speaking in the first half or the first 15 minutes of this presentation is essential, sorta setting the stage. When we talk about 21st century audiology, we have to think about that 22nd century audiologist that's coming and getting trained as of now, so sort of education in light of the changes that are coming along in audiology. So that's why we thought it would be relevant for me to speak a little bit about the challenges and opportunity from an educational perspective. There's a lot of things that's happening obviously from a research, from a clinical perspective, from your classroom perspective. What are the changes that we need to be aware of and how can we make sure, because any time, it's an amazing cycle, any time we as educators think about writing a curriculum or putting together our coursework, we're not thinking about today because the typical student that enters an audiology program, three or four-year track, enters today, means they're not gonna practice until three to four years from now. I mean they're gonna be audiologist in training. So we really have to look three to four years, and sometimes 10 years ahead, to say where do we see this audiologist, how can we prepare them and what are the challenges and opportunities in this? So audiology as a

profession, at least for America, for U.S., we have evolved and philosophically in a way involving, even though the degree is the doctoral degree. AUD is the recognized entry-level degree. From a curricular perspective, I believe we are evolving. We're evolving from the curriculum that was from the master's degree into a doctoral degree curriculum. Even now in several conferences when we sit and have a discussion about are we educating to the breadth of scope of practice? Are the students that are in the program today ready for where we want to see them in a few years? So we're also, philosophically again, evolving from an allied health perspective to an independent practitioner perspective, so the goal for the profession to becoming a doctoring profession, and then also to become the patient's point of entry for hearing health care system. So that was sort of where this whole transition started and we see ourselves as audiologist as your hearing health care, your point-of-entry hearing health care professional.

So in order for all this to happen, where do we see ourselves go? So from a curricular perspective, with this change, the new doctoral level education, we had to define and implement what does that mean? What does that mean to have a doctoral curriculum? Can we take the same courses that was offered at the master's level and add on another year of externship, so make it a three to four-year program? Will that be considered a doctoral level or can we take this opportunity to evolve better courses, to go in depth of things that we were not able to do in a master's curriculum given the limited number of semesters in a year? Are we able to go to a more in-depth curriculum? Can we benchmark ourselves against other doctoring professions? So we had optometry. We looked at medicine from our college perspective as the Osborne College of Audiology. So we looked at other doctoring professions. What do they do? What do they do differently? How are they different from a practice perspective, from a philosophical perspective? And the bigger part is the legislative perspective. I mean what do we need to educate them so that if we have to go for a change in scope of practice or an expanded scope of practice, what should we actually be educating them on? So what does a curriculum look like when the scope of practice has not changed,

but the responsibility for patient outcomes will change? So some of the areas like interprofessional education sort of came about, I mean it has always been there. We never just called it that. We called it sometimes interdisciplinary education, but the focus and the depth in which these gets addressed have actually expanded. So we had to think about everything and the bigger part is we had to figure out, where do we, as audiologist or hearing health care professionals, fit in this big puzzle called a patient? The patient has all these symptoms. Where do we fit and what is our role in taking care of the quality of life for the patient? So in order to be able to do that, what should we be teaching our students? So the challenge of what to learn for an unknown future, are we gonna be the same scope of practice? Are we gonna become expanded scope of practice? So they were. I mean ours is one of these programs where we really taught a lot about the foundations like taking history or the biomedical sciences, histology, pharmacology, like six credits worth of that, and you don't see implementation of that. I mean the students take a lot of hours in pharmacology or biochemistry or histology, sort of similar to other doctoring professions. If you think about it, and if they're not using it on a day-to-day clinical practice because our scope of practice has not expanded, then what justifies that education?

So what should be changed in the curriculum? That is a big challenge that all the academic programs face because we don't know what the endpoint is. So challenges that we face when benchmarked with other doctoring professions are: this model increases workload in general. That is if you're adding all these foundational sciences courses and you cannot apply that directly to the clinical settings, I mean how do students retain that information? So are you giving them all this information that they're not able to use right away? The allied health model of audiology doesn't really let us implement such in-depth knowledge of biomedical sciences. So when the students evaluate this new content, the relevance of the content was proving to be very difficult. So again that unknown future versus how deep should we go into all these areas. So those were some of our challenges, but opportunities, it really gave us the opportunities to include various areas of content that was not a part of the original

audiology degree, I mean in-depth education in pretty much every area that we could dream about. It gave us the ability to expand the breadth and depth of academic and clinical content. The diverse settings that these students are in different placements, every student at least gets four to six different sites of rotations and each site is very, very different. So our goal of putting all this out there in education is that education preceding legislation. So does education really precede legislation? So if we wanna go for expanded scope of practice as a legislative change, we put our curriculum out there, will it stand its own merit? So if we want prescriptive rights, do the students know enough in pharmacology? So the question the legislators ask us is not about, are you gonna give them this further education of all these things? It's rather are your students educated in these areas to practice under this current scope of practice? So this is something that we learned early on, that we believe transformation starts from the curricular content. Leveling of academic programs become really essential, and the outcomes of the program should also be assessed rather using an intense mechanism that has the goal of the future of the profession in public health, and not just based on current scope of practice. This is something as a college, as a program we strongly believe in.

So some of the challenges other than the curricular challenges that we face today in audiology education is the flat applicant pool. I mean not all the programs participate in a centralized application process. However it's really essential for us to know: How can we create this awareness? How can we make the profession attractive enough? Because if the total number of qualified audiologists are reduced or if the number is going down, then we're not able to meet the demands of the population. So obviously we need to see how we can increase the number of applicants for the profession. We did a survey of all the program directors through the CAuDP forum. We actually presented this data at one of the AAAs before and we wanted to know: What do other program directors think? What do other programs base their curriculum on? Do they wanna see the old master's plus a CFY model? Do they see themselves as an allied health profession? So if you see over here, basically you can see that this is your allied

health with the master's plus CFY. The second one is allied health with the physician supervision, with the current scope of practice, allied health with current scope of practice. Next one is expanded scope of practice, limited license practitioner, and then there's just other models that's out there. So you can see that several programs, about 26 of them responded out of the 75 accredited programs and about... You can see over here that about 50% of them really wanted the limited licensed practitioner with current scope of practice. We didn't see a lot of popularity for expanded scope of practice. So 54% of them would prepare graduates for limited license practitioner status under the current scope of practice, 27% of them with expanded scope of practice and 8% of them actually preferred going back to master's model plus a clinical fellowship year, so two years of master's plus one year of fellowship, which this was very surprising to us that we thought everybody was onboard and this is out of the 26 programs that responded.

So what does this mean? So in general education and how we learn and what are the things we want to educate our students in so that they are ready, they are ready to practice in the advancements that are coming, and maybe an expanded scope of practice and what we have to look forward to. So are we preparing them to meet the future? Are we preparing them to be ready for the future and current developments? And then the big role of clinical supervisors, our clinical preceptors in audiology education because about 60 to 70% of the clinical training of any student in any program happens outside the campus when they are on their rotations, when they are on their externship, that full year that they spend. So if the clinical preceptors are not ready to adapt the forward-thinking model expanded scope of practice, then it becomes a student is not really using what they learned in school, overall. So I think as we are going into the next segment of our lecture by Doctor Brenda Ryals, you will see that there's all these advances from the research that's happening which seems like a while. It is happening right now. When do we apply it clinically, but are we ready to learn from that and clinically apply for it? So I think from a curricular perspective, we have a lot of challenges but we have also a lot of opportunities to prepare our students

to meet the expanding need overall even though the future sorta seems a little unknown, but I think we can be prepared for what is to come. So with this, I'm gonna conclude my segment of this to hopefully setting the stage from an education perspective. I would like to introduce Doctor Brenda Ryals who's a professor emeritus of communication sciences and disorders and is also the Director of Auditory Research Labs at James Madison University. So her research has focused on issues of auditory plasticity and the neural and functional consequences of hair cell regeneration and during the development, during the development phases and also after an injury. So her research focuses on understanding the impact of hair cell generation on central auditory mechanisms and hearing in birds and how that can be used for humans, so how to use that knowledge. So in this webinar, talking about the future of audiology, I truly cannot think of a better topic than this. It is my pleasure to welcome Doctor Ryals to share her findings and research and in general her thoughts about what is out there for us in 21st century audiology. Doctor Ryals?

- [Brenda] Thank you, thank you Doctor Aravamudhan. I have a hard time saying your name, Aravamudhan. Thank you for Salus University for inviting me to give this talk and thank you to all of you out there in the audience. I hope that you will enjoy what I have to say. I do want to say that I am speaking today, October 16th, 2019, and the topic that I have is about hair cell regeneration and restoration of hearing and essentially the cure for deafness. So what I say to you today is true for October 16th, 2019. Things can change rapidly in this field, in molecular biology and in neuroscience. So that's just a caveat to say that I hope that if you're listening to this two years from now there are things that have advanced from today, but what we say today is true for today. So why are we talking about this now? Why do you care as audiologist practicing clinicians about regeneration therapies when we know that we can't do this in humans yet? So I imagine you could tell me why, but I put this slide up only because your patients are reading these sorts of articles. This is an article that was in the New Yorker a couple years ago, there was an article this year in the Wall Street Journal, and there's certainly things all over the Internet and on YouTube about curing deafness through these sorts

of pharmaceutical strategies. So we are the professionals that folks come to with hearing loss and we need to be able to be on top of things to tell them where we stand. They wanna know what their options are. So you are there as a counselor. They want to know, are these therapies right for my child? One thing I will say, we're gonna talk about these hair cell regeneration and restoration and your role in the future will be to talk about who's a good candidate for this. And one of the limitations or I guess things that you should think about is that hair cell regeneration in children with genetic deafness is a special situation. So if you have a genetic form of deafness, and we know 50 to 60% of children who have severe a significant hearing loss have a genetic form of deafness, then that's a different kind of therapy and there are tremendous advances being made right now on gene therapy, and even CRISPR technology. We'll talk about that later a little bit to address mutations in genes and replacement of genes and that sorta thing. that's not what I'm gonna be talking about, but you need to understand that you all will be the ones who are helping people decide: Who do you send to a doctor, or to a physician, to whatever therapist is gonna do this procedure. Who are the right candidates? Is it appropriate for genetic deafness? Is it appropriate for infants and children, and what about the elderly? Is it appropriate for long-standing hearing loss? So hopefully I will, that's a goal of mine so that you can answer these questions, but we're gonna start from birds.

What have we learned from birds? So we'll do that fairly quickly 'cause I'm sure you don't treat any birds, but I think it's interesting that we learned things from birds so that we can't dismiss research in animals that are not humans. How does development fit into the therapy procedure for hair cell regeneration and what are the current strategies? When we talk about current strategies, I'm gonna talk about these three strategies. So cell cycle strategies, that has to do with stem cells. Cell fate, how do cells decide or that it'd be hair cells or not? That has to do with gene therapy. And I don't have this up here right now, but it also is cell fate, and that is about small molecule therapy. so there are three main strategies: stem cells, gene therapy and small molecule therapy. Once we got through sort of that background that I do wanna

talk to you about some current human clinical trials and we'll end on challenges for you in audiology and working with your patients. I am completely open to your asking questions while I talk. It's a little difficult to be interactive in this format, but I much prefer that you don't have to wait to the very end to ask questions because you'll forget what you wanted ask. So please feel free to ask a question and I'll try to answer it as we go along. So prior to 1988, the dogma was: when hair cells die, all that remains is a phalangeal scar. You've seen pictures of that on scanning electron microscopy where a hair cell dies from noise or ototoxic drugs and the supporting cells merge to form a scar. The auditory nerve synapses swell and die and those anatomical changes result in permanent sensorineural hearing loss. So I got my Master's degree in Audiology in 1973 before many of you probably were born. That's odd, but anyway, and that's what I was practicing as an audiologist and that's what I know. So why question that? Where do birds come in?

So I did want you to know that I am a clinical audiologist. I practiced clinical audiology for 17 years in a private practice and at the VA hospital before I went back to school to learn more about basic science. The reason I did that was because I was working with infants in the NICU and I got very interested in how hearing develops. So I went back to school at the University of Virginia and I met this gentleman here, Ed Rubel, who had just come from Yale University. He's an experimental psychologist and a neuroscientist and he was very interested in how hearing develops. And he said to me, "If you wanna know how hearing develops, "you should look at birds "because they come nicely packaged in this little shell "and we can get to them at many stages, "embryonic stages of development "and we can look and see what the triggers are "that are causing hearing loss "or causing normal development." So I thought that seemed like a reasonable idea. So I started to look at birds, but I wondered are birds really a good model for humans? What are the similarities and what are the differences? So I wanted you to know, for one thing, that avian ears, birds, their ear develops very similarly to mammals and humans. So this is a panel from an article by Atkinson et al. in Development. I encourage you to use this URL to go there, this URL here, to go to this

article and read about normal development of the inner ear and how that informs regeneration therapies, but this is to show you, this is sort of the gross idea about the development from the otitis. So in early embryonic development we get this sheath of cells, and then those cells are triggered in some way to decide to become inner hair cells or outer hair cells, and then this lovely organ of Corti develops with the pillar cells and this whole organization with supporting cells, and part of that differentiation or a big part of it are the molecular and genetic signals that cause those hair cells to divide and, those cells to divide and become hair cells. So I'm not gonna do a whole lot of alphabet soup, but some of these molecular and genetic signals are critical for you to understand if you wanna have any background in understanding why we are using stem cells or why we're doing gene therapy or small molecule therapy. So we'll go through just these few molecular and transcription factors.

So this is a prosensory cell. That means it's a stem cell that already sort of knows it wants to become a pre-neuron. It's sort of already kind of programmed along this line instead of, for example, an intestinal cell or a blood cell. So we have a pre-neuron and these signals, Jagged, and Sox, Notch, Wnt, and FGF, are all present and allow that cell to be open to triggers to say: divide, don't divide, stop dividing, and then become a supporting cell or a hair cell. We've learned a lot about how we can reintroduce this to regenerate hair cells from this normal developmental process and this process happens in birds, and in humans and in mammals. It's a very conserved process. So this is the situation. We have Notch. Notch is a transcription factor that you, I just mention it because you may have stepped on the tail of a lizard at some point. If you did, you noticed that the tail broke off and you felt terrible, but not too terrible because you knew that tail would grow back. That's because those cells in the tail of the lizard have this signal, Notch, present so that those cells can be signaled. They're open to a signal that says: regrow, redivide, and to the signals that say become tail cells, not heart cells or whatever. So Notch is really a critical factor in cellular differentiation, as is Wnt, and FGF is a growth factor. So all those things are there. This, p27 kip1, we'll talk about a little bit, and sonic hedgehog, SHH. So p27 kip1 is what we call a tumor

suppressor gene. That means that there needs to be some signal that says to those cells: stop dividing, and now differentiate into whatever you're gonna be, a heart or whatever. So p27 kip1 is a tumor suppressor gene. SHH, if you turn that off and you have the presence of p27 kip1, then cells stop dividing. They exit the cycle. So now they're not gonna divide anymore. Will they just sit there and be undifferentiated or will they become a supporting cell or a hair cell? And these are the signals, this Hes1, Hes5, this growth factor that say: become a supporting cell, a nonsensory cell, or in the presence of Atoh1 and Wnt and FGF become either an inner or an outer hair cell. So that ladder of development has become critical in understanding how we might regenerate hair cells when they're lost after damage. So because that's present in birds too, the other thing that's important I think are interesting about birds and why we can learn from them is that their hearing thresholds are similar to humans and other mammals.

So this is a canary, for example. This is absolute thresholds and frequency, and this is humans. We know our best hearing is somewhere between one and 4,000 kilohertz, and so are birds. They're not quite as sensitive as we are, but their frequency of sensitivity is the same as ours. You might have thought that maybe they'd be more sensitive to higher frequencies since they tweet, but in fact if you look at the fundamental frequency for their tweets, they're right in there with 1,000 to 4,000 hertz. So they have comparable best frequencies in hearing and they vocalize for breeding and socialization just like we do. So vocal performance is really critical for them, unlike small rodents, mice, things like that, but they are not exactly like us. So this is a cross-section of the inner ear, auditory organ of the inner ear of a bird. This is a drawing, this is a real cross-section, this is a scanning EOM of a bird inner ear. And what you see is that we don't have that lovely organ of Corti. We have a sheath of hair cells across here with a tectorial membrane. Those hair cells are very similar to our hair cells, except that they are not divided into inner and outer types and there's no pressed in and active process. The tops of the hair cells on the sheath, and as you can see, it's just a small curve, not the two 1/2 curves like coils like we have in the human cochlea. So that's

very different, but the similarity is that the hair cells themselves work as mechanotransducers, just like the hair cells in mammalian ears. So do birds respond the same as mammals to acoustic trauma? And as you of course know, the answer is no, they don't. Oops, I guess I should get rid of that. Huh, is that one? Yeah, oh, there we go, okay. So this is the basal tip of a canary, actually of a canary inner ear after kanamycin. What you see is that there are no hair cells, a few little dead hair cells, but nothing, but if you wait long enough, it repopulates with all of these new hair cells. So, no, they don't respond the same way as mammals. And what we learned is that, so again you have the damaged inner ear here, the regenerated inner ear here. What we learned is that there are precursor cells, these are cells that are already in the avian inner ear that are signaled to restart the developmental mechanism to form hair cells when they're damaged. So in birds hair cells can be replaced through mitosis. So we know, if you look up here, you see, this is a close-up of this area here, so you see this little teeny hair cell here. It's gonna grow and become a real nice mature hair cell. There was a division of supporting cells that created a new hair cell.

So hair cells can be replaced through restarting that mitotic process that we looked at and we saw in the slide on development. There are endogenous precursor cells. Those are supporting cells that already exist in the inner ear of birds that when damaged by noise or drugs, they restart development and begin to divide. So every time you see a red star like this , this could be something you should pay attention to because they'll be a question on your exam at the end. So just a little hint: hair cells can form after damage in birds through mitosis or cell division. Also what we know is that hair cells can be replaced through transdifferentiation or conversion. So we know that there are cells in the inner ear of birds that don't have to divide. They can just be convinced through molecular and genetic signals to become a different type of hair cell, or a different type of cell, to become a hair cell. So there's another way besides mitosis, they don't have to undergo division, some cells can just be signaled to automatically become a hair cell. So if there's damage and you have supporting cells in the inner ear remaining, if we had the right molecular and genetic signals, those cells could be

convinced to become hair cells. So here's what we know. There are supporting cells that are triggered to either re-enter the cell cycle or to dedifferentiate and convert or transform to hair cells. When mature hair cells are damaged or destroyed. That happens in birds automatically. We don't have to do anything, except destroy some hair cells and that happens. We also know that automatically those new hair cells, this is the bottom of a hair cell in a bird that's been regenerated after damage, and we can see the synapse has formed right on that hair cell. So we know they get reinnervated after regeneration and this is an audiogram if you will for a bird. This would be before any damage, then if you use an ototoxic drug, they lose hearing. I did some work with Robert Dooling at the University of Maryland many years ago because we believed that, what we really wanna know and what you all really wanna know is a behavioral response. So instead of using an ADR or some kind of electrophysiological test, we trained birds to respond to tones in a behavioral paradigm.

So these are behavioral audiograms. So this is how the bird heard behaviorally after ototoxic drug, and we know the hair cells were gone. We know it takes about a month to get the hair cells all back and working. This is the hearing after about a month or two. So hair cells reform, they're reinnervated, and the result is restored behavioral hearing. So the first question after we discovered this and published in 1988 with birds was: "Why don't mammals do that?" So the first question would be: are there precursor cells? We know there are supporting cells. Is there something different about the mammalian ear that would say those precursor cells just can't become hair cells? So initial studies were about that and what we found was, this is Patricia White's work out of UCLA, all cells, all cells in the mammalian sensory epithelium, that's in the Cordi or anywhere actually in the scala media, can be induced to become hair cells, which is astounding to me, but it's true only if though, and this is only if they're immature. So where we found this could be true only up to postnatal seven to 10 days in mice. After that in adults, that didn't happen. Also, only if they are released from local signals. So this happened in a dish. You take out the inner ear and you put the pillar cells in a test tube and you put the Deiters' cells. In all the difference cells you can get those cells to

become hair cells, that is gross stereocilia, if they're not next to each other. If they stay next to each other, they have inhibition and they have to have the genetic signal for hair cell differentiation. So what would that be? Well, before I talk about that, let's just say that in mammals there are tumor suppressor proteins that inhibit the cell cycle and growth factors that stimulate reentry. So in a normal or a regular mammal, these tumor suppressor genes, this p27 kip1 is present. It's off during early development, so cells are dividing and filling up the otitis and the organ of Corti area, and then it comes on and those cells stop dividing. The way that we found that, Neil Segil published an article long time ago in 1999 that showed that mice who didn't have this p27 kip1 gene made too many hair cells. So in mammals, that tumor suppressor gene is present and doesn't turn off after damage. It's still there. So we know that there are precursor cells in the mammalian inner ear, supporting cells, that can become hair cells, but they don't through division because there are cell-cell interactions and because of tumor suppressor genes. Those things don't work that way in birds, right?

So if you go back to this picture, you can see that p27 kip1 is on. The cell doesn't wanna divide anymore. It has to have a signal to become a hair cell. So these are the factors that we wanna look at in trying to convince a mammalian inner ear to regrow a hair cell. So hair cell regeneration of birds essentially recapitulates development across all animals and we know that FGF, which is fibroblast growth factor, Notch and Wnt, signaling here and here, are important for differentiation and maintenance. So let's imagine that or let's remember that, and in particular Notch 'cause you see it here and you see it here. It also went in FGF. Those are critical factors in making a new hair cell. So what is the hair cell gene? I said that there has to be some genetic factor. So just because you turn off Notch doesn't mean you're gonna become a hair cell, like in a lizard, you become a tail cell. The hair cell gene, this Atoh1 is both necessary and sufficient for precursor cells to differentiate to hair cells. We discovered this intersophilla in flies when we were looking at mechanotransducers in the vertebrae, the vercelli in flies, intersophilla. We know that this is what makes stereocilia grow and become mechanotransducers. So if you don't have Atoh1, you can't get a hair cell.

That's the hair cell gene. So here's a proposed mechanism for hair cell regeneration without putting in something exogenous. This is endogenous hair cell regeneration. Either you inhibit the p27 kip1. I said when it turned on, you can't divide anymore. Maybe you could inhibit that, use a Wnt pathway, get the cells to divide, and then become a hair cell, or you could inhibit Notch when we turn off Notch and overexpress Atoh1. You could get a cell you just directly become a hair cell. So those are the two critical pieces of information that people have been using to develop their strategies for restoring or regrowing hair cells in the mammalian inner ear. So the next step, let's translate this to mammals. We know that all of the cells of the mammalian cochlea are precursor cells under the right circumstances. Those circumstances would be released inhibition of tumor suppressor genes, for example, or cell-cell interaction signals, or to introduce morphogenetic signals that change cell fate so the cells that remain could be convinced to become a different type of cell.

So we use that information to develop these new strategies because it doesn't happen normally without anything as a therapy. In mammals we need to induce mitosis, so we're trying to talk about stem cells, or we need to regulate cell fate and in that way we could use gene therapy or molecular small molecule therapy. Normally I'm giving this talk and I'm looking at the audience and I can tell if you guys are with me or not, but I can't tell here so I hope that we're come along in the right way and everybody's with me so far. If you have a question, again, feel free to interrupt at any time. Those of you on the West Coast, if you've had your coffee, just interrupt anytime 'cause you're awake now. So stem cell therapy introduces exogenous cells, so cells that are not currently in the damaged cochlea, and they're not inhibited from cell division. So we know stem cells can divide and replicate continuously. So we could introduce stem cells and those cells would divide, we'll talk about that, or we could introduce a factor or morphogen that controls transcription or stimulates the cells that survive to change their cell fate and become hair cells, or we could use small molecule therapy to target developmental pathways. I'll get to small molecule therapy, but if that's not familiar to many of you, you may have heard of stem cell therapy and gene therapy. So small

molecule therapy, many drugs are based on the introduction of small molecules. For example, acetylsalicylic acid is a small molecule in aspirin. So you put together a formula that includes some of these small molecules that signal cells to do things, and that's how you do drug development. So we'll talk about how small molecule therapy is also being used as a strategy for hair cell regeneration. I have a little movie that I wanted to show you. Yehoash Raphael from the University of Michigan shared this with me and his team drew it so I have to give them credit, but it seems to me, it's nice to at least think about in some sort of visual way how you might introduce stem cells and what they would do or how you might use gene therapy.

So if you could play the movie now. And Chuck Berlin did the music behind this, so I have to give him credit too. So here's the organ of Cordi and the hair cells. Something damages them and they die. The cells that remain move down and form up the sheath of cells. At that point, as they are forming that sheath, we could introduce a new cell that would get signals from local cells that suggests it should become a hair cell, we'll talk about how that might work, or we could introduce a virus, a vector, that carries Atoh1 for example into the DNA of the cell nucleus that then signals that cell to become a hair cell. So those are the two primary ways we've been looking at how to produce a new cell, a hair cell, after damage. Okay, thank you. So let's talk about stem cells for a second. We know that stem cells are able to self-renew. So they are able to continually divide. They divide like a mother cell that then divides to become another mother cell and a specific other cell type. So you get this asymmetrical division that continues, and continues, and continues. I put this Irish Stem Cell Foundation. I think that you'll be able to click a link here, but if not you can just go to YouTube and type in Irish Stem Cell Foundation. They have a very nice little video that really talks about stem cells in general if you wanna know a little more about stem cells. So stem cells are an exogenous way you introduce a new cell into the inner ear. And we know that generally the easiest way to work with stem cells is to work with embryonic stem cells, embryonic stem cells. We want those embryonic stem cells to become cells with stereocilia bundles, mechanosensory currents. There have been studies out of many

places, but Stanford University for one. If you go to Stanford website and say regeneration you'll get some interesting papers, but there's a problem as you may think about with embryonic stem cells. So, for example, they're not easy to come by. They're well regulated in this country, in the United States. So if you can't get embryonic stem cells and others cells that are multipotent, so embryonic stem cells are what are called pluripotent stem cells. They have the capacity to become any sort of cell, like blood cell, a heart cell, an intestinal cell, any sort of cell. Multipotent cells are cells that already have started down a line to become a specific kind of cell, like a neuron of some sort or a blood cell, which could be either a platelet or a red blood cell, but they're multipotent.

You can use them, for example, if we had a multipotent preneural stem cell, that might work pretty well in the inner ear. But embryonic stem cells are the most plastic and easy to convince to become a particular cell type, but they're regulated. They're not easy to come by. And so that's been a problem, it was a problem, at least until 2012 when Yamanaka in Japan and Gurdon in the UK were given a Nobel Prize for Physiology and Medicine for IPS cells. These are induced pluripotent stem cells. I used to ask people, I can't ask you all, but raise your hand if you ever heard of Dolly the sheep where they were able to clone another animal from Dolly. The way they did that or were able to do that, so take a cell from any cell, a skin cell, any cell, and you reverse it in time so that it becomes de-differentiated. There are four factors, and I won't go into the whole deal about how this works, but you can take a cell, put it through this four-factor procedure and take it back to becoming a stem cell. So I could use an induced pluripotent stem cell, and I could get that from the same animals, cells in that same animal or the same person and create pluripotent stem cells. So that's a big advance. Those pluripotent stem cells are easy to come by. It takes several of them. It doesn't work 100% of the time, but you can produce a lot of stem cells and that obviates the problems with embryonic stem cells. So as I said, they're available. There is no problem with the immuno-reaction that you might get with other stem cells. They can be abundant. They can be made from humans with the disease or disorder

so they can be pretty specific to the person, and we know that you can use induced pluripotent stem cells to form auditory neurons and hair cells. And then actually these articles have shown that those hair cells not only have stereocilia, but they can create the mechanocurrents, the electrical currents that we need for hair cells to actually work in transducing this to neurons. So IPS cells can be used to create stem cells that will create hair cells and neurons in a dish, in a test tube. We know that if you put human embryonic stem cells from otic progenitors. So they're little preneuron kind of stem cells. Folks in the UK mostly, but other authors as well from these articles, have shown that in living animals after damage you can restore function looking at an ABR and they've shown that some new hair cells appear to be there. This article from 2017, this first article was in an auditory neuropathy model. So you know in auditory neuropathy, you have inner hair cells that are still there, but you have a problem at the synapse level.

And so what they did was take this mouse that had a problem to the synapse level and the inner hair cells and put in stem cells and they got better, improved ABRs. This was sort of replicated in a sensorineural hearing loss model and they didn't get improvement. What really happened we think, mostly, as a general rule, stem cells that are transplanted into the inner ear and into the cochlea tend to migrate to the modiolus and become neurons. So in a auditory neuropathy model, you could see how you could get improved hearing after those stem cells because perhaps they increased the synaptic potentials that were available to the animals. Maybe you got some hair cells, but probably you mostly got neurons. And it seems to be the case, for most of the studies so far anyway, not everyone, but many, using stem cells, they're a little difficult to get in and not damage the cochlea for one thing so it's much easier to put them in the modiolus, for example, but in general they just appear to be driven to a neural fate. So stem cells are best at becoming neurons. A problem, as I mentioned, with stem cells is how do you get them into the inner ear? So some people have thought about using cochlear implants. So we have somebody who's got a significant hearing loss and they're gonna get a cochlear implant. What if we could use those stem cells, put

them in a cochlear implant and grow some new neurons? We think that probably somebody who has a cochlear implant, the more neurons you have, the less juice you'll have to use, less stimulation on the electrodes. And so you could get better thresholds or improved function with less electrical stimulation. So what about that? How would that work? So you'd have to have, this is just an uncoiled cochlea from base to apex, and you'd have to have... You have hair cells. So you need to have some hair cells there, but even if you didn't have hair cells, if you could stimulate the neurons, you'd have to get neurons and they also have to connect to the right hair cell. So the difficulty so far in using implants to grow new neurons is specifying which hair cell and which neuron. So we know we have that place for frequency transformation that happens all the way up to the brain and we don't wanna mess that up. So specifying one neuron to the right hair cell that goes on to the brain has been a problem so far that we still have to work on in order to use cochlear implants to get new neurons that would improve function.

So as I go through here, I wanna talk about what the stem cells can do, I'll talk about gene therapy, and then we'll talk about what the challenges are so that you understand how fast we might move forward with these therapies or what the stumbling blocks might be. Well, first of all, for stem cells, there was a problem with availability, but perhaps the IPS cells have helped with that. Delivery is gonna be a challenge because to get them into the cochlea and specifically they have to be in the organ of Corti and specifically bathed in endolymph and under a tectorial membrane, this could be a challenge if we wanna use stem cells for hair cell replacement. They have to integrate into the side of lesion and we know that endolymph is toxic to hair cells. Only the stereocilia can be an endolymph. The body of the hair cell needs to be in cortilymph or perilymph, whatever you wanna call that, underneath the stereocilia. So that's a challenge, and in general they appear to be driven to a neural fate. So my opinion is that the stem cells will be more successful or we'll see success more quickly with them if we're trying to regenerate neurons. What about gene therapy? Okay, gene therapy. Gene therapy involves introducing genetic material into a person's cells to fight

disease. Gene therapy isn't specific to the inner ear, of course. What we wanna do is take a gene and get into the person's cells to fight the disease, or in the case of hair cell regeneration, to take a cell that's sitting there that survived the damage and convince it to become a hair cell that functions and restores hearing. How do you get a gene into the inner ear or anywhere? Generally genes are delivered with a carrier. That carrier is known as a vector and the most common type of vectors are adenoviruses, or viruses, generally adenoviruses. So a new gene is injected into the person. So let's think about what a virus does. So when a virus enters your system, it immediately goes to the cell nucleus, to the DNA to modify the transcription factor on that cell to tell that cell or to do something bad. So if you take a virus and you strip away the function of that virus, a normal function of the virus, so now that just wants to go to DNA and you add a gene to that, guess what, we would wanna add Atoh1, the hair cell gene. Then we could inject that into the system, into the inner ear, and that gene would go directly to, carried by the virus, into the DNA of the cells that survive and become a hair cell. So that's the theory. That's how gene therapy might work in the inner ear. You can go to this website for NIH. It gives you lots more details about how it works.

So what kind of genetic signals we wanna use? Of course we wanna use signals that transform at this point, that transform this cell that survived to become a hair cell and we kinda would like some supporting cells too. We don't wanna take all the cells and become hair cells, then we don't have any of that beautiful architecture of the organ of Corti, for example. So what we would want is to have Notch and Delta because that's gonna say this cell can change. We want to enhance Wnt, FGF, and certainly Atoh1 if you want a hair cell, and then if you want a support cell, then you wanna enhance these factors. So fairly long time ago, 15, 16 years ago, Doctor Raphael at the University of Michigan in Ann Arbor showed in a living animal, in a guinea pig, that you could use a viral vector that's loaded with Atoh1 into a damaged guinea pig cochlea and get new hair cells in the damaged regions and get improved ABRs. So I don't know about you all, but that was on NPR and it was all over the papers and it was a terrific discovery, and then nothing happened much with it for 10 years or more and the

problem was that these viral vectors, these vectors are not necessarily well homogeneous. So sometimes the vectors don't go to the right places. Sometimes they are good. Sometimes they carry the gene well. Sometimes they don't. People just weren't able to really replicate this study until 2014 when there was a good gene therapy Atoh1 experiment that replicated Doctor Yehoash's study and they showed that you could get increased hair cells, same way in a guinea pig, but the ABR did not improve. So those hair cells were not either sufficient in number or sufficiently innervated to increase the thresholds, to improve thresholds.

So gene therapy in mammals has happened and it's worked, but it hasn't worked consistently. So the challenge, there are several challenges for gene therapy. If you signal the cells that are there to become hair cells, then you will deplete supporting cells or you'll change the mechanics potentially of the inner ear and how the traveling wave works. We know that for some pathologies, gene therapy, for example if the electrical environment of the hair cells is not good, so if the stria vascularis is not working to produce a good endocochlear potential, then it won't matter at all if you get a new hair cell 'cause that hair cell will not create a mechanical current. So we need to have the right people for gene therapy, select the right candidates. Also, I didn't mention this much before, but viral vectors can produce an immune response. You have to be very careful with the virus that you use. We know that those cells have to be innervated. The good news about that is it seems as though when you get a new hair cell, they become innervated. Now whether the innervation is appropriate to the brain, we don't know, but imagine how that would be the case, and timing for introducing the gene could be critical. So in all of the cases so far in animals for gene therapy, the gene has been delivered relatively soon after the hearing loss occurred. So what if you have a long-standing hearing loss? Will those cells be as flexible, as plastic to gene therapy as the cells that are remaining relatively soon after damage? So timing could be critical as well. Maybe work better for sudden hearing loss or for acoustic trauma than for age-related hearing loss, for example. So let's talk about clinical trials because I said that gene therapy and Atoh1, this looks pretty promising and it actually worked in

mammals, at least one time off and on. So there is a human clinical trial currently underway, sponsored by Novartis, which I think is Eli Lilly company but I'm not positive, it is Novartis. And that trial involves injecting a vector loaded with Atoh1, actually it's a human analog of Atoh1, into the inner ear through a hole that's drilled into the footplate as the stapes and that vector carries the promoter gene that confines the Atoh1 to the support cells of the cochlea. You don't really wanna get a hair cell up on the stria, for example. So that's the procedure. Enrollment began in 2014. They expect completion in 2021. They hope to get 45 people with sensorineural hearing loss. The target population are people 18 to, adults, 18 to 75 years old with severe to profound hearing loss and the primary outcome right now anyway is safety, serious adverse events, and they are looking at changes in audiometry and they're also gonna do vestibular test and speech audiometry. So that is underway. We'll talk of little bit more about that later in a little bit, but there is a human clinical trial so far as I know.

Safety has not been a problem, but efficacy and whether things are working and increasing hearing, I don't think they, they've not reported anything on that yet so we don't know. So that's gene therapy. Now let's talk about molecular therapy, small molecule therapy. So in 2013, folks up at Harvard in the mass pioneer labs showed that they could use genes that are important for cell differentiation, those Notch, Sox, those genes, to inhibit the cell fate after damage and turn on the transcription factor that stimulates Atoh1 and results in cells becoming hair cells. So this happened in mice, in adult mice, and they were able to show that they got new hair cells and they got improved ABRs. If you want to go, you can go to, just Google these authors and that they published in Neuron. And there's a lovely little video abstract about the study that Albert Edge, one of the authors, performs, and it's a great little summary of the study and how it worked. One thing, let's see what's next on the slide, okay, one thing to mention about this study, it's an elegant study and it's quite well designed, one of the things people ask me, maybe you're thinking, "So how do we get these vectors into the inner ear? "It sounds like we're having to inject them "directly into the inner ear," and you're right about that. So right now delivery of these drugs or molecules or

genes are being directly delivered either through the middle ear so that there's likely a gel that goes onto the round window, or through a hole in the stapes or in the bony labyrinth. What we'd like, what everyone would like is to be able to take a pill. You wanna have it systemically. So these folks initially thought well we'll try this small molecule so it's sort of a cocktail of factors that you could give as a pill. How would this work? What they found is if you inhibit Notch, this cocktail of small molecules, and you give it systemically, it's generally failed because Notch inhibition, you don't wanna do that in the liver, for example, or other places. You need to direct this small molecule therapy to the inner ear. So this is not gonna work. This right now, this particular method doesn't work as a pill. The other thing, let's see if I can show this, so just to say that there is a clinical trial based on this therapy.

This is the small molecule drug that inhibits Notch signaling. Audion Therapeutics is funding this and it's part of the EU Horizon 2020 funded project Regain. This is the website and it'll tell you all about where they are with their clinical trial in doing that. And they have finished their patient recruitment, so we hope that we will see some results on safety and perhaps efficacy, but mostly about safety very soon. There's a challenge for this approach though. So you remember that in normal development, we had to have Notch, hold on. I'm sorry. This is the problem when you're at work and giving a talk, so just ignore that phone. So the challenge is that Notch, remember we had the turn off Notch so that the cells could be signaled to become another kind of cell or if you turn off Notch, the cells are done. If you turn on Notch, then the cells are open to changing their fate. What we know is that Notch signaling begins to go away early after birth in mice and certainly probably true in humans as well so that if there's no Notch to turn on or off, how will this work? What this article as cited down here showed is that when the folks first did their study with this Notch inhibition, they showed new hair cells at the apex and their ABR showed improved thresholds for low frequencies, low frequencies in mice. So in fact Notch stays around at the apex for a while, but then goes away. So if it isn't available in adults, how could blocking it work? So this could be a challenge. If Notch isn't available, what else could you do? And this

is probably the last therapy that I wanna talk about or approach and that is activating an existing precursor. So these are very interesting little cells that exist in the human and mammalian cochlea. They're labeled Lgr5+ cells. These are supporting cells in the cochlea that normally give rise to hair cells and they are cells that have Atoh1 sitting around them that hasn't gone away in maturity. So they are capable of becoming hair cells after damage in the mammalian cochlea. So instead of forcing a conversion of cells that don't have Atoh1, what if you could trigger these Lgr5+ cells through a Wnt-Notch pathway to proliferate? You would not only convince cells to differentiate, you could actually have the cells that are there already, these Lgr5+ cells, divide and become hair cells and be in a situation where the cell-cell signals would prevent them from continuing continuous division. So that could solve the problem or the challenge of cell depletion or supporting cell depletion.

So there is a clinical trial using that approach in humans. Frequency Therapeutics is funding that. They're testing the safety of their drug. So their drug is sort of a cocktail. They have a Wnt activator. There's some vitamins in there, some growth factors in there, several things that are in FX-322 that are proprietary of course, but they are doing a double-blind placebo-controlled single-dose study through intratympanic injections. So there they put their drug on a sort of gel that sits on the round window and is slowly released. They have finished their recruitment, I believe, and so far, at least in the first part of phase one, safety has not been a problem with doing that. We still don't know about efficacy, whether it works. So this is a summary of the current clinical trials. We have the Regain one which is a Notch inhibitor by Audion Therapeutics. Something happened here, did I do that? Okay. We have the viral vector Atoh1 therapy that's funded by Novartis that has several sites now at University of Kansas, and Columbia, and NYU, and we have the small molecule therapy from Frequency Therapeutics using FX-322. So there are three clinical trials currently, as of October 16th, 2019 in humans for hair cell regeneration. There are actually 42 if you count 'em clinical trials in humans to protect or prevent hearing loss that don't involve necessarily hair cell regeneration. They involve other factors, other things that are

going on, there are a lot of clinical trials, more on protection than on regeneration. These three are the ones that are currently active on regeneration after damage. This is again a summary. So this Notch inhibitor has positive results of the first 15 patients. The adenovirus vector for atonal transcription is complete, part A and D, so it's safe. Part C enrollment is open. That's to see if it really works to improve hearing and the FX-322 has safety reported as okay, but we don't know anything else about that, except that it's safe right now. A very short mention about clinical trials 'cause it sounds very exciting that we have human clinical trials on hair cell regeneration. The caveat here are the challenges that only about half, a little more than half, of clinical trials advanced from Phase I to Phase II. So many, many of the drugs that go in are safe, but they don't show enough efficacy to go into Phase II to see if the does would work. Only about 10% of the Phase X trials, Phase I, sorry, Phase I trials advanced to approval.

So this the bad news about clinical trials and while you hear about people talking about clinical trials and how expensive they are, and in fact many many or most Phase I clinical trials don't result in effective treatment and drugs that are on the market. One thing though that will increase that 10% to 25% is finding biomarkers, and that's where audiologist I think can come in and help here. If you can stratify patients, if we can pick the right patients, we already know that hair cell regeneration is not gonna help somebody who has no endocochlear potential. So can you think of a patient who has no endocochlear potential or a bad endocochlear potential? The first thing that comes to my mind is connexin 26. They have a potassium recycling problem. It's a genetic problem. It's not gonna help to regenerate a hair cell in those patients. So if we can help physicians identify patients where hair cell regeneration will help, that will increase the probability that clinical trials will work. This is my summary slide. We know translation from chickens to mammals is progressing rapidly. It's been 30 years, but hey, 30 years, that's not too bad. We're working to overcome the challenges, talked about the challenges. We know that at least for birds where regeneration occurs naturally and within days or weeks of hair cell loss that auditory perception recovers.

So we've done some experiments that show, not only does the audiogram recover, but if you will speech perception, so perception of bird song, complicated, complex signals, they recover their ability to understand and know that, and vocal production is only temporarily affected. So finally in the last, I guess I have a few minutes, how long do I have? 15 maybe, 15 more minutes? Let's talk about what your role is as an audiologist in all of this. So first of all, appropriate counseling. Hopefully I've met my goal here and you'll be able to tell your patients when will hair cell regeneration be a reality? Not tomorrow. You need to help yourself right now and that's not gonna happen now, but in my opinion, it will happen. It's simply a matter of time. Advances occur every day. Talk to me again in 10 years. Will hearing aids or cochlear implants continue to be necessary in the face of hair cell regeneration? Certainly. We know that regeneration may not give us the whole inner ear back of course and probably to regenerate a cell you'll have to have some sort of procedure to inject whatever the therapy is into the inner ear and probably hearing aids or cochlear implants will work together with regeneration. In my view, that's the most likely scenario for right now.

The other thing that audiologists are gonna do and I mentioned before is the termination of candidacy. Where is the site of lesion? What's the underlying pathology? We're pretty good at being able to tell the difference between conductive and sensory neuron hearing loss and we're not too bad at being able to tell whether it's a hair cell or a neural problem. We've got OAEs and we have ADRs. We can do pretty good at that, but we're not so great at being more specific about endocochlear potential, for example. Is that the underlying reason we don't get an ABR or an LAE? Maybe we'll be involved with gene chips so that we can see whether connexin 26 is the problem or is there a genetic problem here that's not syndromic that we can address differently. We need a test to differentiate inner hair cell from VIII nerve problem synapses. Maybe the hidden hearing loss research that's going on now will help us develop such a test or a battery of tests. We need to test for endocochlear function. If it's just for those folks with connexin 26 we can use a gene chip, but maybe other people are having endocochlear function. We know for example from animal studies that one of the

factors that seems to be an issue as we get older is that the endocochlear potential decreases, and then finally we are gonna be the people who quantify therapeutic success. So how will we know whether this thing worked? Will we be doing ABRs? Will we be doing OAEs? We'll do pre-and postoperative or pre-and post-therapeutic testing. What will be the right test to do? And I think that we should be involved, we will be, we should be involved in determining outcome and using the right test and telling the people who are providing the therapy whether it worked or not. I'm open to questions now. I'm happy to take questions. I would say that now that we know this can happen, we'll never go back to thinking it can't happen. So in my view, regeneration and restoration of hair cells and neurons will happen in humans. It's a matter of time. Just give us a little more time. Okay, questions? Oh, I see now, okay.

So Carolina has a question. She says, "What's the loss degree "and age of the patients in the trials?" So that varies over the trials. So all of the trials that I mentioned are in adults. And in fact one of the trials, the Atoh1 Novartis one is on adults up to the age of 85, but none of the trials are children. I guess Caroline you also asked: "Is speech discrimination also tested "to check improvement or just threshold?" So at this point, only one trial where they describe the trial on clinicaltrials.gov, only the Atoh1 gene therapy approach is using speech discrimination or speech perception, but their patients have severe to profound hearing loss so they don't have much very good speech perception. So I think that in most of the trials, we'll be talking about people who have moderate to moderately severe or profound hearing loss. So that could be a limitation in getting a good change in speech discrimination if we have a floor effect. Mostly it's just thresholds as this point. Woops, I had a question. I lost it. Can you get it back for me? It went away. Sorry. I think it was from somebody in the South Carolina. If there aren't any questions, I have a couple comments that I didn't have a chance to really talk about that I'll share with you since we've got a few more minutes. One is tinnitus. So sometimes when I give this talk, people ask me about tinnitus. Is regeneration a therapy that might work for tinnitus? So no, it is not. We think that tinnitus right now and our understanding of tinnitus is it's neural in origin and not

confined to the cochlea. So unfortunately patients with relatively good hearing but just tinnitus as their primary complaint are not candidates at this point for this sort of therapy. I can go through a couple: so if your patient says to you, "Will this therapy work for me? "Can I take a pill?" When they develop it. So the first question, will this therapy work for me, that's where audiologists will be involved. I would repeat to you that most all of the animal studies have been done relatively soon after the hearing loss. So if you have a progressive or a long-standing hearing loss, we are less sure that it will work, but certainly noise-induced hearing loss or sudden sensorineural hearing loss, acquired hearing loss, that is more likely a good candidate at this point. Genetic deafness is not a candidate for this sort of therapy, but there are tremendous strides being made in therapies, pharmaceutical approaches, biologic approaches for genetic deafness. You probably remember or maybe you read about the TMC1 discovery. The TMC1 gene is the gene that allows the stereocilia to be mechanotransducers. So that's critical. If you have a genetic problem with that gene, then you could use gene replacement therapy to address that and a couple of studies have been done on that particular gene and mutations in mice to show that it could work. So yes, but that's a different kind of therapy.

It's gene therapy, but it's not gene therapy to regenerate hair cells. It's to replace a defective gene. One additional question, okay. Jenelle asked, "What's the best source to direct patients to "who are interested in this, "either for curiosity or for participating?" That's a good question. I would say if you go to the clinical trials, then you could, .gov, and go to those three clinical trials, you could go to Novartis, or Audion, or Frequency Therapeutics, and they could probably direct the patient to understanding what they're doing. There is a consortium, trying to think of the name of it now, maybe you can email me. Let me give everyone my email. Maybe I'll type this in here. Can they see this? It's... Yeah so Christie's gonna share my email with you all. There's a consortium working on, it's the Hearing Loss, HLA, Hearing Loss Association. They give grants and they have a consortium working specifically on hair cell regeneration. I would direct patients there, but I'll have to look up the website for

you. The last thing I might mention because I think that you may have heard of it, and maybe some of your children, patients with children, would wanna know about this, so that's about cord blood. There has been a patient trial on using cord blood to get restoration of hearing on acquired hearing loss in children. Cord blood has the stem cells in it. This was a small study. If you wanna look it up, it's in by Baumgartner in the Journal of Audiology and Otology in 2018. There were I think 11 subjects, not quite half, I think five out of the 11 subjects. First, there was no danger. So it was safe. They had acquired hearing loss, so they were born with normal hearing, and then they acquired hearing loss for various reasons. The kids who were in the study were between I think six months and six years and they got a very small, less than 10 dB improvement in hearing. So if they talk about cord blood, I would suggest that you go to this article. If you write me, I can give it to you again, but it's on whether banking cord blood, if your patient wants to bank cord blood if that's a reasonable idea because it's quite expensive. Okay, anything else? Is this the program? Yes, thank you. Giovanna just found the website for me. It's, let me see if I can print this out for you. hearinghealthfoundation.org/hearing-restoration-project. That's a great website. That's a great site for patients to go to. Thank you, Giovanna.

- [Moderator] Thank you Doctor Ryals. That concludes today's webinar. We appreciate your time and your expertise and we wanna give a big thank you to Salus University as well for inviting Doctor Ryals on. I hope everyone has a wonderful day and thank you for tuning in.

- [Brenda] Thank you.