Claire Schultz: Thank you for taking the time to join us for this special research update. Gathered for the live presentation today are members of the Hearing Health Foundation board of directors, staff, and some of the Hearing Restoration Project consortium scientists.

For some of you this is an introduction, while others have heard a little about Hearing Restoration Project (HRP) from a colleague, friend, or loved one and are participating today to learn more. Some of you have a deeper level of understanding of our research and looking for an update on hair cell regeneration, HRP progress made to date and where we're heading in the months ahead. No matter who you are, we all have one thing in common, a personal connection to hearing loss.

Our goal is that everyone who listens to the presentation will obtain new information on hearing loss and our research toward a cure. It doesn't end today. If after listening to the briefing, time passes and you have questions or thoughts, we are your source for reliable, accurate, up to date information. We intend to have many more special research updates with our researchers in the future, and there will be plenty of opportunity for learning as we go forward. Thank you again for your time and interest in our Hearing Restoration Project.
Overview

- Welcome & Introductions
- History of Hearing Health Foundation
- The Challenge
- HRP Consortium History & Model
- Approach to Hearing Restoration
- This Year’s Projects
- The Next Five Years
- Q & A
Claire Schultz: Hearing Health Foundation was founded in 1958, almost 60 years ago, and has established a reputation for pioneering breakthroughs in hearing and balance research.

Some examples of this include:

- We were early supporters of the revolutionary cochlear implant, and today over 220,000 children and now adults are benefiting.
- We advocated for the passage of *Universal Newborn Hearing Screening* legislation in the 1990's. Today, 97% of newborns are tested for hearing loss at birth.
- The *Emerging Research Grants Program* provides seed funding for researchers in hearing and balance science such as discoveries in hair cell regeneration, tinnitus, hyperacusis, and Meniere’s research. Many of these researchers have had fruitful careers and gone on to obtain federal funding for their work; this is one of our measures of success.

**Today:**
Focused on finding a biological cure for hearing loss and tinnitus through hair cell regeneration!

Experience throughout the years leads us to the focus today; to find a biologic cure for hearing loss and tinnitus through hair cell regeneration, though our Hearing Restoration Project.
The Hearing Restoration Project

Peter Barr-Gillespie, Scientific Director of the HRP, to introduce the HRP, the motivation behind it, the approaches that it’s taking, and progress to date.
Peter Barr-Gillespie gave an overview of the ear and its anatomy. The above slide is a cross-section of the external ear.
The above slide shows the outer, middle, and inner ears. The internal ear is made up of two divisions:

1. Vestibular system, used to give your sense of position of your head in space.
2. Auditory system, which is responsible for conducting sound.
   - The yellow area illustrates the auditory system, which includes the middle ear (transmits sound) and the cochlea (the snail-shaped structure where the hair cells reside).
On the left is a rough sketch of the ear.

In the middle is a cross-section through the part of the inner ear that is responsible for detecting sound, the cochlea; here you see a complex system of multiple chambers.

- The cells colored red are the so-called hair cells, named for their cluster of cilia—not because they are related to hair on the skin. Hair cells are the sensory cells that respond to sound and are also the cells that are uniquely vulnerable to damage.

- There are other cells around the hair cells—supporting cells—that I will say more about later.

On the right, we see that the hair cells have a very specialized component at the top of the cell called the hair bundle—this is the antenna that responds to sound. We think that when the ear is exposed to noise, the hair bundle can be damaged and the hair cell then often dies.
This is a picture of the top of one hair cell (in green)—with the hair bundle on top. The cell itself goes down deep below the plane of the picture. The cell is surrounded by other cells, the so-called supporting cells. These cells are crucial because we think they may have the capability of turning in to hair cells. We already know that's true in some species.
As mentioned earlier, hair cells can be destroyed by noise.

Above, you can see a picture of a cochlea. The white V-shaped structures are hair bundles of the cochlea hair cells.

You can see that after noise, many of the hair bundles are missing completely and that in some cases, the hair cells actually have died. Likewise, other treatments, conditions that lead to loss of hearing, like some types of antibiotics, some types of cancer chemotherapeutic agents, and even just aging leads similarly to the loss of the hair cells.

Losing hair cells means losing hearing.
So, in the past century, the primary treatment for hearing loss has been hearing aids and cochlear implants. And these have been very successful, but certainly are imperfect.

I think for this century that we have got a number of different ways for getting into more effective therapy.

1. **Preventing the damage to the hair cells in the first place** is one of the best things that you can do to preserve your hearing and we need to continue to tell that to young people in particular.

2. There are approaches going on for **gene therapy**, particularly for people who have lost hearing due to genetic disorders.

3. The majority of people who have lost hearing have done so through noise damage or aging, and they might be candidates for turning the hair cells back on, **restoring the hair cells, regenerating the hair cells. And that's the focus of the Hearing Restoration Project.**
The Hearing Restoration Project was founded just a few years ago; we are the only consortium that is focused on hair cell regeneration.

There are 14 different investigators, plus myself, scientific director.

One of the key things about the consortium is that we are a collaborative group. We share our data, not just the data that are funded by the HRP, but other data as well. Even more importantly, we share our ideas. The ideas that one lab has might stimulate some exciting research in another lab and the fact that we can get together and discuss these ideas is truly stimulating.

The HRP can also fund projects that don't necessarily fit into the goals of the NIH yet are still essential for hair cell regeneration. That has been a value-added role for the Hearing Restoration Project.

Finally, I just want to point out that we have a Scientific Advisory Board (SAB) consisting of a number of different scientists from across the country. The SAB gives us oversight; they evaluate the HRP proposals and they give us guidance as to directions in which to go.
There are 15 of us in the HRP from across the United States and, in fact, international. There is one member from Canada and one member from the UK, and we brought together the best of the auditory field in order to bring our minds together and be able to do things that we can’t do on our own.
One of the key facets of the Hearing Restoration Project's approach is that we use three different animal models for studying hair cell regeneration. We do this for a couple of different reasons, highlighted on the following slides.
First of all, two of those models, the chick and the zebrafish, show robust hair cell regeneration.

If you damage the hair cells of a chick or a fish, within a short time, only a day or two for the fish, a few weeks for the chick, the hair cells come back; new hair cells are formed.

So, that's spectacular, because it tells us that animals are capable of regenerating hair cells.
By contrast, the mouse is our other experimental model, and it stands in for people. Like in the human, the mouse shows no hair cell regeneration after a few days after birth.

You can damage the hair cells in the mouse and as far as we can tell, nothing much happens in terms of restoring hair cells. So, if we can figure out how to regenerate hair cells in the mouse, then we will be able to regenerate hair cells in people.
Here’s a more detailed picture of the inner ear.

There is a couple of features here that will give you an idea of how we're trying to go about this project. Here you can see both hair cells in red and supporting cells in yellow. The supporting cells surround the hair cells, playing a structural role as their name would seem to indicate.

Neither the hair cells nor the supporting cells are homogenous. There are two types of hair cells, one type that transmits sound information to the brain and the other that plays a role as an amplifier. There are also different types of supporting cells that play different roles in the ear—you can see that their shapes vary.
We know from experiments done in fish and chick that damage to hair cells leads to supporting cells giving rise to new hair cells. Either a supporting cell can turn directly into a hair cell, or it can divide and make one hair cell and one supporting cell.

However, in the mouse, that doesn't seem to be the case. Supporting cells don't convert to hair cells after the hair cells are damaged.

So, we're interested in understanding what the molecular signatures are of the supporting cells.

• How do supporting cells that can turn into hair cells differ from those that cannot turn into hair cells?

• What are the molecular differences?
  • Those are things that we potentially could tweak.

• Also, what happens to the supporting cells in the mouse after damage to the cochlea? If the whole cochlea degenerated and we are left with nothing, then it would be much more difficult to stimulate regeneration of hair cells.
More specifically, we’re interested in knowing whether after damage to the hair cells, does the cochlear repair itself? Do specialized supporting cells remain?.

In the bottom right panel here is a "flat epithelium", which sometimes happens after hair cell damage; if this is the prominent response, we think that these cells will be harder to turn into hair cells than those represented by the color yellow.
The purpose of the HRP is to ask can we stimulate or inhibit regeneration pathways within the cells to make new hair cells. By pathways, I am referring to sequences of molecular reactions that take place in the cell.

There actually are hundreds or even thousands of pathways that are operative simultaneously in a cell. We think that they're likely to be a few of those pathways that are important for hair cell regeneration. Maybe in the mouse there are pathways that inhibit regeneration, and we need to interfere with those pathways, or maybe there is a pathway that is active in chick and fish, but is not active in the mouse, and we have to stimulate that pathway.

One way or another, we want to convert the supporting cells or remaining cells into hair cells by overcoming whatever the block is to regeneration.
Our strategic plan, which has been developed over the years by the consortium members working together, consists of three separate phases.

We have made a lot of progress on **Phase 1** and we have initiated **Phase 2**.

1. In Phase 1 we compare fish, chick, and mouse and see what happens to their supporting cells after hair cells are damaged -- we want to find out if the cells remain behind or we have the flat epithelium. In addition, we will see if we can identify pathways that are stimulatory or inhibitory for regeneration.

2. Phase 2 is crucial. The experiments that come out of the Phase 1 are not definitive. We need to evaluate the pathways we have identified. We might get hundreds of leads from Phase 1, but we need to test those molecules and pathways in our model systems to figure out which of those pathways are relevant for hair cell regeneration.

3. Finally, in the Phase 3 of the strategic plan, we will move towards developing therapies and treatment. Using the model systems in the mouse that we developed for Phase 2, we will screen for drugs that trigger hair cell regeneration.
One of the most important sets of experiments we have done is called genomic profiling -- we want to find out which genes are turned on or turned off in response to damage to hair cells. In particular, we want to understand why the mouse doesn't show the same type of responses as the fish and the chick.
We have made a lot of progress with this step. We have generated large data sets from each species. We have done multiple types of experiments with each species and used a technique called bioinformatics to try to understand the relevance of the data that we have generated.

Just having large lists of genes is not terribly informative. We need to understand which of the genes play together in a pathway, and which of the genes might be relevant for regeneration.
We need to understand which of the genes play together in a pathway, and which of the genes might be relevant for regeneration. And so these bioinformatics experiments are crucial for that.
The other part of Phase 1 is to understand the supporting cell stage. Do we get supporting cells remaining or a flat epithelium?

I would say that our experiments look pretty good. They suggest strongly that at least in mouse, many months after damage to the hair cells, highly specialized supporting cells remain. It corroborates data gleaned from studying human ears, which suggests the same. So, this is important because it suggests that we can target these supporting cells, which on a molecular level are much more closely related to the hair cells than these flat epithelium cells.
In Phase 2, we will modulate the genes of interest and ask whether we can turn on regeneration in the mouse, or maybe turn off regeneration in the fish or chick.

We have got a variety of new projects that are starting on Phase 2, and I'm going to briefly talk about the projects that are funded for this year in a couple of slides.
So, we haven't started Phase 3 yet. We really need to get a healthy way through Phase 2 for two reasons.

1. We need to make sure that we have the ideal model system for carrying out the experiments which are going to be time-consuming, expensive, and technically challenging.

2. We need to be sure that we know what we're targeting, because the Phase 3 experiments could be more directed if we're pretty sure we know which pathways are important.
A lot of what we have done so far to date really has depended on the ability to work together. We have an annual meeting for a couple of days and we have another meeting associated with the Association for Research in Otolaryngology (ARO) meeting, which all of the HRP scientists go to. Moreover, we talk on the phone at least monthly and oftentimes much more than that. Together this allows us to continuously collaborate, sharing ideas, and it is really delightful from a scientist's point of view.

- We avoid repetition of experiments. Instead, we can assign an experiment to a specific person.
- Collaboration also allows us to do multi-species, multi-model experiments, and that really couldn't be done in any one lab. That's really crucial to our approach, that I think it has been very successful so far.
- Allows us to see the new data months or even years before you would if we had to wait for it to be published. And the collaboration is working.

One measure of our ability to collaborate is to look at who comes together to propose projects to be funded by the HRP within our group—almost all of the projects involve multiple scientists, usually from different institutions, coming together to propose experimental approach.
Progress on Phase 1:

- We've identified a variety of candidates for these molecular triggers of hair cell regeneration and the pathways that are necessary.
  - We have too many, so we really are continuing to use bioinformatics methods to glean through and determine which are most important.
  - We have definitively shown, at least in the mouse, the specialized supporting cells remain.
  - We know now what our target cells are for triggering hair cell regeneration.
Phase 2 has begun, but we haven’t stopped Phase 1:

- We’ve got multiple approaches to try and see whether or not we can block regeneration in fish and chick or stimulate regeneration in mouse.
And Phase 3 is really, truly is in sight, although I think there is still plenty of work to be done on Phase 2.
1) **X Cells**: Multiple scientists independently saw the same unexpected observation, which we realized in our fall meeting. We found that when hair cells were damaged in the mouse, weeks to months later we saw cells in the ear that looked like they were hybrids between supporting cells and hair cells. This observation suggests that in the mouse, some supporting cells do respond to damage and begin to turn into hair cells.

   • What the experiments seem to be telling us is that supporting cells have responded to the damage to hair cells in the mouse. They have gone partway along the pathway towards making a new hair cell, but they’re stuck somewhere. And we would like to know where they’re stuck because that would be a target for doing some sort of molecular manipulation to allow further hair cell regeneration.

   • This project illustrates the value of the consortium and the collaboration amongst the consortium members. A multi-investigator project was proposed and we have fast-tracked funding for this project.

2) **Bioinformatics**: We have several projects designed to help understand the abundance of data.

3) **Discovery science** projects to help understand at a detailed level how hair cell regeneration works in the species that show it. Or how it doesn't work in the mouse. We have clues that are pointing us in the right direction towards overcoming it, but we have a lot more to learn.
4. Phase 2 just began and we only have a limited amount of funds for these projects. They have started screening some of the molecules and pathways but also developing tools and techniques that allow us to do this better in the future.

- **Functional Testing** is a project that couples together fish and chick and mouse and asks for a gene that we can identify in those three species that might be playing a role in regeneration.

- **Mouse Model** focuses on killing the hair cells cleanly to be analyze what happens to the supporting cells over time.

- Researching **Gene Delivery** methods in mouse – may lead to a source of genetic modulation of the pathway rather than a drug modulation for Phase 3.

- **Mouse Functional Testing** key candidate molecules using a novel set of techniques.

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### 2015 Funded Projects

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We've made a lot of progress with a relatively small amount of money.

1. We have learned a lot from Phase 1, and we're going to continue Phase 1 because there are many more molecules and pathways to identify.

2. The Phase 2 experiments that we are doing now are laborious, one molecule at a time. It will take time to get through large numbers of molecules or pathways.

3. Phase 2 must be scaled up. This will require quite a bit more investment into the HRP. We need to be able to test many more genes and to test combinations of different genes, and we also need to develop better methods for screening genes and eventually drugs.

4. One approach to scaling up would be to develop a cell line that replicated hair cell regeneration in vitro, although this is not easy to do. It is crucial before we turn into Phase 3 to make sure that we have the right model for screening drugs or genes, if that is the approach we go.
We have learned over the past 25 years or so that the hair cell regeneration is indeed a plausible way in which to eventually treat hearing and balance disorders. It will not give us hearing restoration next year, but it will eventually.

I also want to point out this is a wonderful time to be a scientist, because the amount of information that we have about how cells operate, not just in the ear but elsewhere in an organism, is extraordinary and we are learning more and more every day and very creative people are developing spectacular technologies that allow us to do experiments that we could only dream of in the past. It's really a fantastic time to be approaching a difficult problem like this.

So, as the slide says, I think the question is not if we will regenerate hair cells in humans, but really when.
Brian Pollard: Question on dimensions of the synaptic -- auditory impact may occur there and I wonder if your project comprehends that problem.

- What I didn't talk about is what the hair cells do after they detect sound, but of course they transmit their information to the auditory nerve, via synapses which are connections between hair cells and the auditory nerve.

- Work primarily coming out of Charlie Lieberman's lab in Boston has shown that noise damage that is reversible from a threshold point of view, noise damage to animal that seems to recover, can lead to irreversible loss of some of the synapses.
  - There is a recognition that a substantial fraction of hearing loss may be due to damage to the synapses.
  - The HRP doesn't address restoration of synapses directly. There are other groups and companies that are working hard on trying to come up with methods to enhance synaptic connections after damage.

- We're striving for a more severe type of damage, and I think the jury is still out as to whether or not the most hearing loss that occurs amongst American population is due to loss of hair cells or loss of synapses.

- Both are surely important, but at the moment, we have limited resources and limited band width, and therefore, we have decided to focus very much on causing regeneration of hair cells.
Who actually owns the patents and rights to the final product when and if you do actually are able to come to some conclusion on this that it actually works?

This is something for the future, as none of the research/work we’re doing now is patentable. When we get to the point where we’re screening drugs, then the Hearing Health Foundation's interest is not necessarily in holding the patents, but rather facilitating the research to make sure that we get to a point where we have a therapy much more quickly than we would otherwise.

And I think that the universities of the scientists are going to be interested in having a role. But I also think that one of the things that I have not mentioned is that we anticipate as we get closer to having actual therapy, or having drugs that we can look at, that we're going to be need to be partnering with industry, and because they have the resources, the big resources, once a lead compound has been identified, to move something forward. So, like I said, it is not the intention of the Hearing Health Foundation to obtain a patent, but, again, just to facilitate the science and the progress towards hearing therapy.

Claire Schultz: In closing, if there are no other questions, our goal going into today was to allow everyone to obtain new information about hearing loss research toward a cure progress to date and looking forward.

What I would say is after listening and while time passes, if you have questions, thoughts, things come to mind, don't be afraid to contact us. We are the source for reliable, accurate, up to date information, in particular in the area of hair cell regeneration.

As I said at the start, we will be holding many more of these special research updates with our researchers. There will be other opportunities to continue to learn as we go and ask questions as we go forward.

Thank you very much for your time and participation today. We hope you got a lot out of the presentation. And we look forward to being in touch with you soon and for you to be in touch with us if you need anything from us. Thank you and have a good afternoon.