Hi everyone, I am Nadine Dehgan, CEO of Hearing Health Foundation. I wanted to thank you all for joining us today for special research update by our Hearing Restoration Project's scientific director, Dr. Peter Barr-Gillespie.

A little background on me, I joined HHF in January as CEO, but had previously consulted with the foundation prior to my accepting my current position. I have a very personal connection to HHF's mission; my father has adult-onset tinnitus (often debilitating) and my brother has had hearing loss since childhood. My youngest daughter, now 4, had painful, recurring ear infections as a baby and toddler that led to residual hearing loss. I am personally motivated to find better therapies and cures to enhance the lives of my loved ones, as well as millions of individuals living with hearing loss and tinnitus.

Joining us today for the live presentation are members of the Hearing Health Foundation board of directors, staff, and other Hearing Restoration Project consortium scientists, as well as longtime supporters of HHF. Thank you for your continued support and interest in HHF and our groundbreaking research.

Our goal is that everyone who listens to the presentation will obtain new information on hearing loss and our research toward cures and treatments. 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 more webinars with our researchers in the future. Thank you again for your time and interest in our Hearing Restoration Project.
Continued

Nadine Dehgan:

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 adults are benefiting.

• We advocated for the passage of the universal newborn hearing screening legislation in the 1990’s, and now ~97% of newborns are tested for hearing loss at birth.

• Our Emerging Research Grants program has and continues to provide seed funding for early stage scientists in hearing and balance science. Many of our funded researchers have gone on to obtain federal funding for their work and this is one of the measures for success.

• Experience throughout the years leads us to the focus today: the Hearing Restoration Project consortium. The consortium is tasked with finding and developing effective therapies for hearing loss and tinnitus through hair cell regeneration.

I would like to now introduce Dr. Peter Barr-Gillespie, Hearing Restoration Project's scientific director and Professor of Otolaryngology at Vollum Institute at Oregon Health and Science University. Dr. Barr-Gillespie will now present on the HRP's plans to develop effective therapies for hearing loss and tinnitus.
**Overview**

- HRP personnel update
- HRP refresher
- How the HRP operates—a year in the life of the HRP
- Progress to date: Two vignettes
- Plans for 2016 and beyond

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**Dr. Peter Barr-Gillespie:**

Thank you, Nadine. I will first provide a quick overview of what my presentation is going to encompass. Next, I thought it would be interesting to present a year in the life of the HRP, how the investigators get together, and how we discuss projects and develop projects.

I'll go on next to giving a couple of vignettes about some ongoing science that's very exciting and conclude with a brief discussion of our plans for 2016 and beyond.
Dr. Peter Barr-Gillespie

I'd like to announce to those of you that don't know that we have a new HRP member, Ronna Hertzano, from the University of Maryland. Ronna is a clinician as well as a research scientist, a rare combination and an asset for the HRP. She has a clinical practice and is a surgeon who focuses on diseases of the ear and the lateral skull base.

Ronna also has a very active research program which focuses on hearing restoration, genetic causes of hearing loss, and mechanisms of control of genes that are important for hearing. Importantly, she has developed a platform, a bioinformatics platform called gEAR, which the HRP benefits from.
Dr. Peter Barr-Gillespie

To provide you with a brief understanding of gEAR, which is an acronym for, gene Expression for Auditory Research, I give you this slide pictured above.

Without worrying about the details, the common thread here is that by using the gEAR, individual gene entries in our complex genomics experiments, which may include 20,000 different entries, can be extracted and then compared against similar entries of the same gene in different data sets. Those who know about the HRP know we're comparing expression of genes from the mouse, zebrafish, and the chick. gEAR allows us to compare a single gene, such as Sox2 (above), and learn about the similarities and differences between the different species and model systems that we're using. We're really excited to have Ronna on board.
HRP Refresher
The Hearing Restoration Project (HRP)

- **Founded in 2011**, HRP is the first international research consortium focused on hair cell regeneration
- Consists of *14 investigators* plus a scientific director
- Overarching principle is **collaboration**: open sharing of data and ideas
- Funds projects that are not traditionally funded by the NIH (e.g., genome-wide inquiries)
- The **Scientific Advisory Board (SAB)** provides oversight and guidance to the HRP Consortium

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**Dr. Peter Barr-Gillespie**

The HRP was founded almost five years ago, and the HRP consists of 14 hair cell regeneration investigators plus me, as the scientific director. We actively collaborate, share our data, and our ideas. This allows us to make progress more quickly than would occur when each individual investigator is locked up in their home department, at their home university.

So we cross those barriers between institutions and allow for collaborative research. The HRP funds projects that are not traditionally funded by the NIH, for example, those large genomic intersects that I referred to earlier.

We also have a Scientific Advisory Board composed of scientists in our field, and they provide an oversight rule and also guidance to our consortium.
Dr. Peter Barr-Gillespie

Some of you may know that the sensory cells of the ear are located deep within the internal ear. There are two main components to the inner ear, both of which signal to the brain; the vestibular system detects head movements, and the cochlea that detects sound.
Dr. Peter Barr-Gillespie

Above is a picture of the cochlea. Each of these white spots that you see here are a hair bundle, the sensory organelle of the ear; each hair bundle projects out of the sensory hair cell. This is what you want your cochlea to look like.
Dr. Peter Barr-Gillespie

Unfortunately, age or noise exposure, as well as to exposure to ototoxic drugs, can all damage the hair cells in the cochlea (the auditory system), leading to a situation seen on the right. The fewer cells one has, the less functional the auditory system is.
Dr. Peter Barr-Gillespie

So the HRP is taking advantage of information that we've had for some time, which is that there are species that show robust hair-cell regeneration as well as those that don't.
Dr. Peter Barr-Gillespie

Unfortunately, humans don't show hair-cell regeneration in the cochlea. However, the chick and the zebrafish show robust hair-cell regeneration after damage.
Dr. Peter Barr-Gillespie

But like humans, mice don't show hair-cell regeneration, at least in the cochlea. They show limited regeneration in the vestibular system, which we also take advantage of.
After hair cells are damaged and die, there are two possible outcomes for the cochlea and the epithelium (cell layer) that imparts its function. Surrounding the hair cells are key cells called supporting cells, which can turn into hair cells after damage in fish and birds (but not mice). In some cases, the epithelium repairs itself, leaving functional supporting cells, but in other cases, the epithelium degenerates and the cells that are left are not the specialized supporting cells.
Dr. Peter Barr-Gillespie

The goal of the HRP is to grow back hair cells either from the repaired epithelium that contains supporting cells or from the flat epithelium. We must manipulate pathways—sequences of reactions that allow cells to control their structure and identity—in order to regrow hair cells. Depending on what prevents hair cell regeneration in mice, we either need to stimulate pathways that are not functional in mice (but are in fish and birds) or we need to inhibit new pathways in mice that prevent the regeneration program from functioning.
Dr. Peter Barr-Gillespie

The Strategic Research Plan has three phases in it.

1. **Phase 1**: To carry out discovery research where we find those pro-regeneration or anti-regeneration pathways by determining what happens in the cochlea after damage. This requires experiments where we damage the hair cells and look across the genome, 20,000 different genes, to see what happens to the gene expression, either in an organism that shows regeneration or an organism that doesn't.

2. **Phase 2**: Once we've identified these pathways, we move to phase 2, where we verify that those pathways are present in the different model systems and prove whether or not the hypotheses that were generated in phase 1 are correct. In phase 2, we also develop assays that allow us to validate these pathways.

3. **Phase 3**: The assays developed in phase 2 will then be used to screen for drugs that would promote hair cell regeneration in the mouse.
Continued from pg. 15

Dr. Peter Barr-Gillespie

Thus far, we have been working on phase 1; however, in the last several years we have moved into phase 2. We will continue to conduct research in phase 1 throughout the life of the project.

One of the things you learn in science is that the more you learn, the more there is that you don't understand. But at the same time, technological advances and advances in understanding of cell biology and other areas mean that every year our ability to determine what's going on in the molecular level gets better and better. Thus, new and expanded understanding of cell and molecular biology means our research in phase 1 will continue. At the same time, we have already made headway and already moved in pathway validation, phase 2.
How Does the HRP Operate?
Dr. Peter Barr-Gillespie

On a yearly basis, there are a number of activities that we carry out.

• We have monthly (sometimes more frequently) phone calls where we discuss projects and share data. At each meeting, two or three of the HRP members give brief presentations about their progress on their funded proposal.
• Those presentations lead to vigorous discussion within the group, which helps us decide which direction to go next with the project and leads to cross-fertilization between projects.
• In addition, many HRP projects are multi-investigator and often multi-institution, and HRP members working on those projects talk independently of our group calls. HRP members talk together very frequently!
A Year in the Life of the HRP

The fall HRP meeting
Held in Seattle in late fall over two days

• Working dinner Sunday:
  • Re-assessment of the Strategic Research Plan
  • Other topics
• Discussions on Monday:
  • Brief collaborative meetings
  • Short research updates
  • Review HRP scientific progress over last year
  • Critically, decide where we are going over the next year
• The upcoming year’s plan on Tuesday:
  • Discuss new projects
  • Assimilate into the Research Plan

Dr. Peter Barr-Gillespie

• A key event in the HRP is the fall meeting. This is a two-day meeting, typically held in Seattle, where we have a working dinner the first night, and then dive into great detail the next day about the operation, goals, and projects of the HRP.
• This is crucial, because all of us are brought together in the same room and we have time to discuss progress, ideas, and come up with plans for the future.
• On the full day of our meeting, we have collaborative meetings among the groups, research updates, but most of all, we review our progress and decide where we're going over the coming year.
Dr. Peter Barr-Gillespie

Coming out of the fall meeting, HRP investigators develop proposals for next year's funding. Ideas are brewing at the Fall meeting, preliminary suggestions are discussed there, and then between people by e-mail or phone. These then coalesce into one-page pre-proposals that all HRP members discuss. *This is a crucial step.* We put our ideas down on paper and chew them out, choose them between all of us.

Some ideas end up getting dropped, as some don't fit into the current research plan. Others are combined so we have larger coordinated activity.

So based on our discussions, each of the projects, which typically includes multiple investigators, submits a full proposal. These proposals are evaluated by all HRP members who are not on that proposal. So everybody gets and gives feedback, and returns it to me.

I then present the full proposals with HRP's evaluations, as well as my own evaluations, to the SAB. Once the SAB comments, sometimes they request more information from the investigators. In those instances, investigators prepare rebuttals that are then re-evaluated by the SAB. So this step is a really important one, having these outside scientists look objectively at the work, which ensures that HHF funds the best possible science.
A Year in the Life of the HRP

The winter HRP meeting at ARO

- Co-incident with the Association for Research in Otolaryngology (ARO) Midwinter Meeting
  - San Diego or Baltimore
- Four-hour dinner meeting:
  - HRP members and Scientific Advisory Board attend
  - Formal and informal opportunities to discuss HRP goals, aspirations, and sticking points
  - Select research presentation updates
  - Open-mic unrestrained discussion

Dr. Peter Barr-Gillespie

We have a second meeting in February, which coincides with a larger meeting that almost all of the HRP members go to—the annual meeting of the Association for Research in Otolaryngology (ARO), which rotates between San Diego and Baltimore. HHF hosts a working dinner in the beginning of the conference.

SAB members are also invited to this HRP meeting at ARO, and many of them come. It's important for us because it allows the SAB members to comment on where we're going and leads to plenty of active discussions amongst the HRP and SAB.
Dr. Peter Barr-Gillespie

So as you know, we have funded a variety of projects in 2015, and these are wrapping up right now.

Many of you know about a very exciting project, the X-Cell project, which was initiated by six of our investigators who jointly recognized that in the mouse there were cells that were seemingly responding to damage to hair cells. These cells had characteristics both of hair cells and of supporting cells, suggesting that supporting cells may be partially turning into hair cells. This was a very exciting result that came out of the 2014 meeting in Seattle, and led to a proposal and a quick funding of the project to study these cells in more detail. This is a good example of how our science works and the power of collaboration.

The group was able to characterize these X-Cells. They found two populations. One population was very interesting but the cells were rare enough that it was difficult to characterize them. The other population did not seem to be supporting cells turning into hair cells.

The interesting cells were unfortunately too rare and to difficult to study, so the group decided to stop the project and write up a summary of what they found. We had hoped for a bigger impact from this project, but that's how science goes—you need to give a project your best shot and see what comes of it.
Dr. Peter Barr-Gillespie

As you can see, there are a number of other phase 1 projects that are going on. The chick utricle response to damage and zebrafish single cell response I will be speaking on in the subsequent slides. I'll also allude to some of the work that's done in the P1 cochlea & mature utricle project.

But suffice it to say that we're making progress both in bioinformatics analysis of our datasets, and also in characterizing another level of regulatory molecules called micro-RNAs (small ribonucleic acids that control expression of genes), and we'll know more about those soon in a project that is still ongoing.

As you can see we are continuing to work on phase 1, and there are many unknown questions about how hair cells and supporting cells respond to damage that may require more discovery research.

That said, we moved into phase 2 with a number of projects in 2015, including one (Raible-Groves-Segil) that uses all three of our model systems to compare different pathways to see if there's similarities between fish and chick and differences with the mouse. This project will continue on for 2016, as they've made significant progress and really provided a couple of models for testing pathways.

There are several other phase 2 projects that are ongoing, and there are some new ones that are going to be funded for 2016 as well.
Dr. Peter Barr-Gillespie

In this part of the presentation, I'd like to switch to giving you a couple of vignettes about some ongoing work in the HRP. I'm going to give you two vignettes.
Dr. Peter Barr-Gillespie

The first one is work that is being carried out by Neil Segil and Andy Groves; their hypothesis is that genes important for regeneration in fish and birds are still present, but they have been turned off as the mouse ear develops. In particular, the structure of the genes has been altered preventing expression. If the gene structure was reverted back so that the genes could be turned on, maybe we could overcome a block to regeneration?
Dr. Peter Barr-Gillespie

This requires first a refresher from basic biology. Remember, that each of the 23 pairs of chromosomes that we have in humans has a variety of levels of structure that, as you go from different scales, changes in different ways.

Moving from the bottom of the slide, with very large organizational structures of the chromosomal DNA, down to the top of the slide, you see the individual DNA strands (the blue strings). At this level, you can see that the DNA strands are wrapped around proteins called histones. Histones compact and control the access of the DNA to regulatory molecules that controls the activity of the genes.

Chemical modifications to histones control how accessible the gene is and hence how much it is turned on. We have very sophisticated assays that allow us to recognize those modifications, by looking directly at chemical modifications that the cell makes to the histone proteins.

Neil and Andy are interested in histone modifications that either turn on or off gene expression. They're also interested in the overall structure of the DNA around the genes of interest, because when the gene is actively turned on, the DNA is made much more accessible. They use a different technology to allow them to measure this accessibility.

And we have really remarkable technology that allows us to look at both of these facets of each gene across all 20,000 genes in the genome.
Dr. Peter Barr-Gillespie

I'm not going to show you all 20,000 genes, but I will show you one—a really important gene called Atoh1, the master regulator of hair cell formation.

A gene has to turn on Atoh1 to become a hair cell, and some types of cells in the inner ear can be forced into turning into a hair cell just by expressing Atoh1.

Above on the left is the linear stretch of the gene itself, and on the right the parts labeled A and B are the DNA stretches that are used to control expression of the Atoh1 protein.

As you can see, the H3K27Ac modification is much more prevalent in hair cells than in supporting cells (green). This is an example of a modification of histones that activates a gene. You can also see that the ATACseq traces (blue) indicate that the accessibility of the Atoh1 gene is greater in hair cells than in supporting cells.
Dr. Peter Barr-Gillespie

The key question for Neil and Andy is—*which genes have their histone modifications or accessibility change as mice lose their ability to regenerate hair cells?*

We take advantage of the fact that immediately after birth, at postnatal day 1, mice can regenerate hair cells in the cochlea, but within five or six days, that ability is lost.

So we compare the epigenetic marks of the genes that are expressed in the inner ear, especially in supporting cells, at the postnatal day 1 (early) and postnatal day 6 (late). Some of the genes that are turned off may be essential for hair cell regeneration.

The next question will be—*can we reverse those changes? And if we do, will that allow hair cell regeneration?*

Those are big and challenging questions to address, but this is an important step because by controlling gene expression, we may be able to control hair cell regeneration.
Dr. Peter Barr-Gillespie

So in the next vignette, I'm going to present a little bit on how we're getting better resolution of events from analyzing signal cells rather than large groups of cells in the ear.

This is work that's carried out by Stefan Heller and Tatjana Piotrowski, and they're carrying out very similar experiments in collaboration.
Dr. Peter Barr-Gillespie

They're taking advantage of modern technology, which is spectacular.

They can take a zebrafish or chick ear and pick out single cells, then use advanced technologies that allow them carry out an analysis of what genes are expressed in hundreds or even thousands of cells.

The pattern of expression of those genes in those individual cells tells us what the cell is doing at this point in time.

For example, if you analyze several hundred cells from a chick vestibular organ, you get figures that look these.

The figure on the left allows us to see the similarities between different cells. Each of the dots corresponds to a single cell, and you can see, there are a bunch of cells that are similar to each other here and a bunch of cells that are similar to each other here; other analyses show that one group is the supporting cells and the other group is the hair cells. The figure shows that the gene expression patterns of the two types of cells are distinct.
Dr. Peter Barr-Gillespie

The key experiment in these single-cell approaches is to carry out damage to the organ; in these experiments in the chick, the hair cells are killed using aminoglycoside drugs, which are ototoxins (ear killing). The orange cells are the cells isolated before the damage, while the blue cells are the cells isolated after damage.

There are interesting new clusters that appear in these damaged ears, and one I'll call your attention to, one is gene expression pattern of the cells is similar to supporting cells (red circle). While most of the genes are expressed in a pattern like supporting cells before damage, there are a few genes that are expressed like they will be in hair cells. One is shown on the right—CALB2. See that the upper left shows that most hair cells express CALB2 (orange to red). Most supporting cells do not express CALB2 (gray). But these special cells (red circle) are close to the supporting cells but express high levels of CALB2.

We think that these are supporting cells that are starting to convert to hair cells, although we have much more analysis to do on them.
Dr. Peter Barr-Gillespie

There are very similar experiments done in fish. This approach is very exciting because it allows for resolution of changes in gene expression in cells very soon after damage; we will presumably be able to follow how the gene expression changes during the whole hair cell regeneration process.

We want to know what happens immediately after hair cells are damaged, before they die. Even more importantly, we want to know how supporting cells respond with gene expression changes in response to the damage. Fish and birds go through with hair cell regeneration, so we want to know the entire sequence of events that is necessary. Mice do not carry out hair cell regeneration, so we want to know where that pathway is blocked.

Genes that are essential for hair cell regeneration in fish and birds that are inhibited in the mouse are then particularly good targets for manipulation.
So where are we going next?

I want to reemphasize that **phase 1** will continue, as technology evolves and we learn more from other areas of science.

That's one of the beauties of science. Everything builds on everything else.
That said, we've got active phase 2 projects going on now.

We've got some exciting new results in the chick and in the fish using gene knockout experiments in the fish, using something you may have heard of, the CRISPR gene modification system, which will allow us to test which pathways are important for regeneration.
Dr. Peter Barr-Gillespie

We're now moving to scaling up phase 2, because we need to be able to screen many genes, because many candidates will come out of phase 1. And we need to be able to screen them in combination.

In 2016, there will be a project that will culture cells from the ear; if successful, this project will allow us to screen for drugs that can cause supporting cells to divide and grow or even to turn into hair cells.
Dr. Peter Barr-Gillespie

And then in the future, still, is the drug screening step, but I think, as I've mentioned before, the screening model is really, really important. And that is something that we're actively working on in phase 2.
Dr. Peter Barr-Gillespie

So in summary, I would say that triggering hair-cell regeneration remains a very promising strategy for eventual treatment of hearing and balance disorders.

It's, of course, more challenging than we thought it was starting out because the system is more complex than we thought. But, we're making significant progress.

The advancements in technologies means that we have a greater and greater ability to study hair-cell regeneration in the different model organisms.

And finally, I think the comparison between the model organisms and the sharing of ideas and many other facets of the collaborative interaction model mean this has been already a fruitful approach and will remain fruitful in the future.
Dr. Peter Barr-Gillespie

So in summary, the question is not if we will be able to regenerate hair cells, but when.

We've made a lot of progress and it's just a matter of time and investment in our model, our collaborative model and other ways of study hair-cell regeneration.
Nadine Dehgan

Thank you, Peter. We appreciate your work and dedicate to HRP and providing all of us with this wonderful update.

I want to thank Laura and Veronica here at the HHF office for making today’s webinar possible.

So our goal today is to allow everyone to obtain new information about hearing loss. We will send out the briefing to everyone who participated, so you can review it again at your leisure, and please feel free to reach out to myself or Peter with any questions.

Thank you very much, Peter. And to all of you.
**Q:** What does HRP know about stem cell research for hearing regeneration at other, non-HRP research centers?

**A:** I think there are multiple ways in which stem cells are going to impact hair cell regeneration—but I’m not yet convinced they are the one true way to finding a therapeutic intervention. They are having in impact immediately because of our ability to turn stem cells into hair cells in vitro; work from a number of groups, most notably Eri Hashino’s, has shown this in a compelling way. These in vitro hair cells allow us to screen pathways and drugs in a far faster way than is possible using tissue. We cannot yet make a cochlea in the dish, but we certainly make hair cell organs that look more like vestibular organs.

The next way stem cells may be important is by stimulating stem cells in the ear, maybe in the adult ear, to turn into hair cells. Here, we don’t isolate the stem cells (except experimentally, to characterize them); we would provide some sort of treatment then to trigger them to proliferate and then turn into hair cells. The work of Albert Edge (HRP member) and Alan Cheng in characterizing the so-called Lgr5 cells fits into the definition of stem cell work, and is quite promising. Indeed, what the HRP discovers may be ways in which to activate Lgr5 cells to proliferate and differentiate, assuming they exist in the adult. This is definitely a promising approach.

The third way stem cells might be used is to grow hair cells in vitro, and then transplant them into a damaged ear. I am not optimistic that this strategy will work; the fluids of the ear are not particularly hospitable to cells, and nobody has shown convincing integration that leads to any recovery of hair cells, let alone function. Still, many people are working on this approach, and someone might be successful. Of the HRP members, Stefan Heller works on this approach, although not with HRP-funded projects.

In the HRP, we had to focus, and we decided that triggering hair cell regeneration in situ was more promising than isolation of stem cells and conversion into hair cells in vitro. We may be proven wrong, but to date I feel comfortable that we’re on the right track.

**Q:** Can you give us an idea of the "manpower" pursuing these projects e.g. are the people mentioned devoting full time to these projects or is it one of many?

**A:** The HRP funds a relatively small fraction of the research activities in each lab. Neil Segil’s lab (DNA structure) is supported extensively by the HRP, but the HRP support of work in the labs of Drs. Groves, Heller, and Piotrowski is much less extensive. Each lab, including Dr. Segil’s could advance much faster with greater HRP support; these projects are limited by the available funds.