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TITLE: Development of New Therapies that Stimulate Hair Cell Regeneration

PRINCIPAL INVESTIGATOR: Dr. Brandon Cox, Ph.D

CONTRACTING ORGANIZATION: Southern Illinois University School of Medicine

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14. ABSTRACT

One of the primary causes of hearing loss, which is prevalent in military service members, is the death of auditory hair cells (HCs) due to chronic or excessive noise exposure. Since HC regeneration was discovered in non-mammalian vertebrates 35 years ago, much progress has been made in understanding the mechanisms that are essential for the transdifferentiation and proliferation of supporting cells. However, this research has failed to translate into clinically meaningful treatments in humans or other mammals which lack the ability to regenerate cochlear HCs. One of the major hurdles is finding appropriate screening models for novel compounds. Previous work has often relied on cell line-based screening, however cell lines fail to accurately represent the complex milieu of the cochlear epithelium and what few *ex vivo* explant or *in vivo* studies have been conducted have often relied on the presence/absence of HCs as an indicator of regeneration. However, this metric cannot accurately identify acute HC protection from actual regeneration of new cells. Therefore, the goal of the proposed studies is to use an improved, more definitive, drug screening method to evaluate a targeted library that was generated by artificial intelligence in hopes of identifying drugs that may induce mammalian HC regeneration. Year one focused on screening compounds using a cochlear explant model that uses fate-mapping to identify regenerated HCs.

15. SUBJECT TERMS
Hair cell regeneration, drug screening, fate-mapping, hearing loss

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1. INTRODUCTION:

Hearing loss commonly occurs after exposure to intense or prolonged levels of loud noise which are inherent to military settings. These noise exposures damage and kill sensory hair cells found in the cochlea of the inner ear, resulting in permanent hearing loss. Regeneration of auditory hair cells naturally occurs in non-mammalian vertebrates such as birds, fish, and amphibians with new hair cells derived from neighboring supporting cells. This occurs by two mechanisms: 1) direct transdifferentiation where supporting cells change cell fate and convert into hair cells and 2) mitotic regeneration where supporting cells proliferate and one or more daughter cells convert into hair cells. While many studies have investigated mechanisms to stimulate hair cell regeneration in animal models, there has been little progress in the development of compounds with regenerative properties. This could be attributed to drug screening methods which only quantify hair cell numbers and thus cannot distinguish between hair cell regeneration, and acute protection or repair. Therefore, the goal of this project is to use an improved, more definitive, drug screening method in which supporting cells and supporting cell-derived progeny are permanently labeled with a fluorescent protein for fate-mapping. This enables us to clearly identify any hair cells that arise after treatment with a candidate compound via regenerative mechanisms by their co-expression of the fluorescent reporter and a hair cell marker. Our screening will be performed using a proprietary library generated from literature reviews combined with artificial intelligence computer-based learning which will enhance the likelihood of identifying drugs that may induce mammalian hair cell regeneration. Aim 1 will screen compounds using a cochlear explant model and any validated hits will be formulated for local inner ear delivery, and *in vivo* pharmacokinetic and initial safety profiling in Aim 2A. Compounds with adequate pharmacokinetic and safety profiles will then be evaluated in Aim 2B for initial *in vivo* efficacy using an adult mouse model of noise-induced hearing loss in which supporting cells are fate-mapped to confirm their regenerative efficacy *in vivo*. Compounds that show evidence of regenerated hair cells in Aim 2B will undergo further testing using a wild-type rat model of noise-induced hearing loss and a larger sample size with multiple cohorts. In year 1 of this award our focus has been on drug screening using the cochlear explant model. We have screened 9 compounds in Aim 1A (to assess toxicity), one compound in Aim 1B (to assess response after hair cell damage), and five compounds in Aim 1C (to identify regenerated hair cells using fate-mapping).

2. KEYWORDS:

hair cell regeneration, drug screening, fate-mapping, hearing loss

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: *In vitro* drug screen using cochlear explants.
Specific Aim 1A: *In vitro* drug screen using cochlear explants from C57Bl/6J mice.
Goals for Y1 were:

1. Task 1: Obtain institutional and DOD approval of the animal protocol for all Aims. Timeline: months 1-4

a. Completed in Y1, Q1. The Southern Illinois University School of Medicine (SIUSOM) IACUC approved our animal protocol #2022-097 on 27-JUN-2022 and the USAMRMC ACURO approved the protocol on 16-AUG-2022. The Otonomy, Inc. IACUC approved our animal protocol #FP071122 on 11-JUL-2022 and the USAMRMC ACURO approved the protocol on 07-SEP-2022.

2. Task 2: Screen ~80 targeted compounds using cochlear explants created from wildtype C57Bl6/J mouse pups under naïve conditions. Timeline: months 4-24
    a. 10% complete. We have tested nine compounds at on a log scale (1-6 doses) to assess for hair cell toxicity and the presence of extra or ectopic hair cells.

Specific Aim 1B: *In vitro* drug screen using cochlear explants from C57Bl/6J mice that have hair cell damage induced by neomycin. Goals for Y1 were:

1. Task 1: Screen compounds that pass Aim 1A using cochlear explants created from wildtype C57Bl6/J mouse pups treated with neomycin to induce hair cell damage. (estimate 90% will pass Aim 1A which is 72 compounds) Timeline: months 6-28
    a. 5% complete. We tested one compound to assess the impact on hair cell numbers after neomycin-induced hair cell damage.

Specific Aim 1C: *In vitro* drug screen using cochlear explants from *Prox1^{CreERT2}:: Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice that have fate-mapping of supporting cells and hair cell damage induced by neomycin. Goals for Y1 were:

1. Task 1: Screen compounds that pass Aim 1B using cochlear explants created from *Prox1^{CreERT2}::Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mouse pups treated with neomycin to induce hair cell damage. (estimate 25% will pass Aim 1A which is 20 compounds) Timeline: months 8-30
    a. 15% complete. We are in the process of testing five compounds to assess whether regenerated hair cells are formed after neomycin-induced hair cell damage.

Specific Aim 2: *In vivo* drug screen using fate-mapping and noise exposure.

Specific Aim 2A: Perform formulation work and *in vivo* inner ear pharmacokinetic and safety profiling on compounds identified in Aim 1C. Goals for Y1 were:

1. Task 1: Perform formulation work on compounds identified in Aim 1C. Timeline: months 9-31
    a. Not started yet. None of the compounds we have tested so far in Aim 1C have produced enough regenerated hair cells to advance to Aim 2.
2. Task 2: Perform pharmacokinetic profiling on compounds identified in Aim 1C Timeline: months 10-34
    a. Not started yet.
3. Task 3: Perform safety/toxicity profiling on compounds identified in Aim 1C that have acceptable pharmacokinetic profiles. Timeline: months 12-36.
    a. Not started yet.

Specific Aim 2B: *In vivo* drug screen using *Fgfr3-iCre^{ERT2}::Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice that have fate-mapping of supporting cells and hair cell damage by noise exposure. Goals for Y1 were:

1. Task 1: Backcross *Fgfr3-iCre^{ERT2}::Rosa26 $^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice with CBA/CaJ mice to remove the *Ah1* locus. Timeline: months 4-6
    a. Not started yet. Since none of the compounds we have tested so far have been advanced to Aim 2, we have delayed this task.
2. Task 2: Screen compounds that pass Aim 2A using 3 month old *Fgfr3-iCre^{ERT2}::Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice exposed to noise to induce hair cell damage. Timeline: months 12-36
    a. Not started yet.

Specific Aim 2C: Repeat the *in vivo* drug screen using wild-type Sprague-Dawley rats and hair cell damage induced by noise exposure. Goals for Y1 were:

1. Task 1: Screen compounds that pass Aim 2B using 3 month old Sprague-Dawley rats exposed to noise to induce hair cell damage. Timeline: months 15-36
    a. Not started yet.

What was accomplished under these goals?

Specific Aim 1A: *In vitro* drug screen using cochlear explants from C57Bl/6J mice.

Aim 1A is a primary safety/toxicity screen with secondary endpoints of defining the appropriate dose for subsequent Aims and evaluating initial efficacy for some compounds. We have generated cochlear explants from wild-type C57Bl6/J mouse pups at postnatal day 2 (P2) and tested nine compounds at a log-scale concentration range (1-6 doses per compound) under naïve conditions (Table 1). Explants were equilibrated overnight, followed by treatment with the candidate compounds for 96 hours. Fixed tissue was then immunostained with the hair cell marker, myosin VIIA, and imaged using confocal microscopy. Qualitative assessment of hair cell morphology was used to identify doses that are toxic to hair cells or if additional hair cells were produced. Note that all compound names use a blinded code to protect intellectual property rights and future patenting ability.

Compound Oto-39 did not cause toxicity to hair cells within the tested dose range and was advanced to Aim 1B. Oto-33 was toxic to hair cells at 50µM, as well as at the lowest dose tested (50nM), but not at 5µM or 500nM, suggesting a biphasic response (Figure 1A-B). We will advance Oto-33 to Aim 1B for testing at the two safe doses. Oto-24 was toxic to hair cells at 100µM, but not at 1µM. We plan to test the 10µM dose before making a decision about whether to move this compound forward. Three compounds (Oto-13, Oto-38, and Oto-49) produced extra, ectopic hair cells that were either lateral or medial to the organ of Corti and were advanced to Aim 1C (Figure 1C-E). We have a small sample size for the three concentrations of Oto-69 and need to create additional explants before making our assessment. Similarly, we have only tested 1 dose of Oto-10 which showed some hair cell loss in the basal turn. We plan to continue experiments with

this drug and try lower doses. Compound Oto-9 was toxic to hair cells at all doses tested and therefore has been excluded from further testing.

Table 1: Compounds tested in Aim 1A

Compound	Mechanism of Action	Doses tested	Outcome
Oto-9	Analog of retinoic acid	5nM, 50nM, 500nM, 5μM, 50μM, 500μM	HC toxicity at all doses tested
Oto-10	HDAC inhibitor	1μM	In testing
Oto-13	DNMT inhibitor	1μM, 10μM, 100μM	Produced extra HCs at 100uM & advanced to Aim 1C
Oto-24	HDAC inhibitor	1μM, 100μM	In testing
Oto-33	Tyrosine kinase inhibitor	50nM, 500nM, 5μM, 50μM	No HC toxicity at 500nM & 5uM. Advanced to Aim 1B.
Oto-38	Rho-kinase inhibitor	100μM, 500μM, 2.5mM	Produced extra HCs at 100uM & advanced to Aim 1C
Oto-39	AhR Ligand	1nM, 10nM, 100nM	No HC toxicity at any dose. Advanced to Aim 1B.
Oto-49	Sonic hedgehog activator	50nM, 5μM, 50μM	Produced extra HCs at all doses & advanced to Aim 1C
Oto-69	AhR Ligand	0.1μM, 1μM, 10μM	In testing

Abbreviations: Aryl Hydrocarbon Receptor (AhR), DNA methyltransferase (DNMT), Histone Deacetylase (HDAC), Hair cells (HCs).

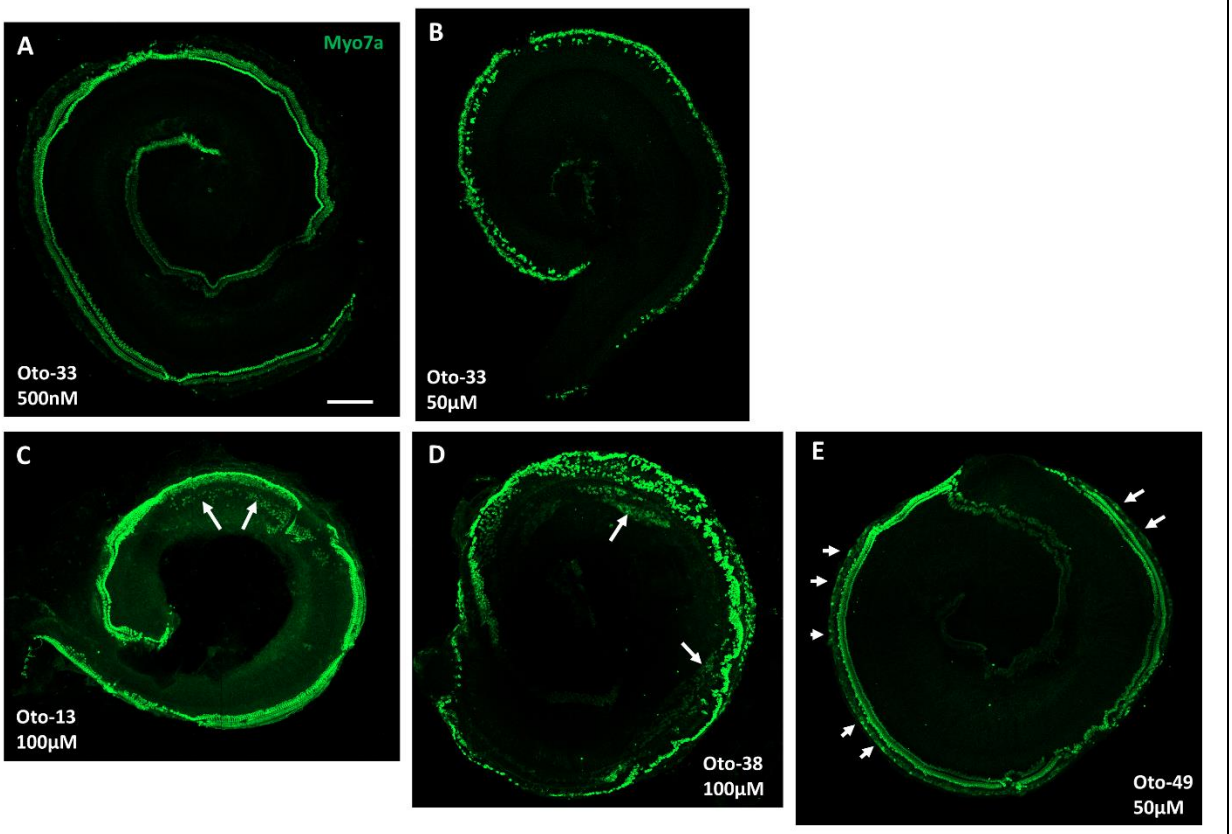


Figure 1: Representative confocal images of cochlear explants from wild-type C57Bl/6J mouse pups treated with four different compounds. Hair cells are labeled by myosin VIIa (Myo7a, green). **A, B,** Oto-33 was toxic to hair cells at 50µM, but not at 500nM. **C, D,** Ectopic hair cells are present medial to the organ of Corti (arrows) in explants treated with Oto-13 and Oto-38. **E,** Ectopic hair cells are present lateral to the organ of Corti (arrows) in explants treated with Oto-49. Scale bar = 200µm.

Stated goals not met: Aim 1A is behind schedule. Since Otonomy, Inc. no longer exists we are completing all analyses at SIUSOM and therefore it is taking more time than originally planned. We also had new staff join the lab when the grant started and there was a training period that slowed our progress in the beginning few months.

Specific Aim 1B: *In vitro* drug screen using cochlear explants from C57Bl/6J mice that have hair cell damage induced by neomycin.

In this Aim we are assessing the ability of each compound to increase hair cell numbers through regeneration or protective/repair mechanisms. Using cochlear explants from P2 wild-type C57Bl6/J mouse pups, we induced hair cell damage by treatment with neomycin for 24 hours. After washes in media, the explants were treated with the candidate compounds that passed Aim 1A for 96 hours and fixed tissue was immunostained with the hair cell marker, myosin VIIA. Using confocal microscopy, we quantified the number of inner and outer hair cells in the middle and basal turns of the

cochlea (two, 200um regions in the middle and basal turns of the cochlea) and compared them to explants that were treated with media alone or neomycin only.

In year 1, we tested one compound (Oto-39) in Aim 1B and found a significant increase in the number of outer hair cells in both the middle and basal turns of the cochlea compared to neomycin-treated controls (Figure 2). This increase was 35% in the middle turn and 41% in the basal turn and thus meets our predetermined criteria to advance to Aim 1C. We also plan to test a higher dose (1µM) of Oto-39 in Aim 1C.

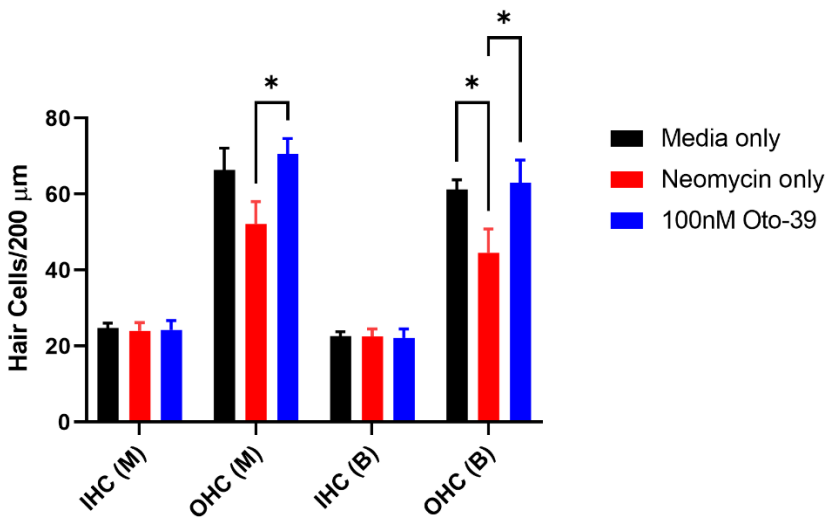


Figure 2: Quantification of hair cells from cochlear explants created from wild-type C57Bl/6J mouse pups that were treated with media (black bars, n=7), 600µM neomycin (red bars, n=11) or 600µM neomycin followed by 100nM Oto-39 (blue bars, n=4). There was a significant increase in the number of outer hair cells (OHCs) in the samples treated with Oto-39 compared to neomycin-treated controls. *p<0.05 as determined by a two-way ANOVA followed by a Tukey's post-hoc test.

Stated goals not met: Aim 1B is behind schedule. We are again performing all analyses at SIUSOM which is taking an increased amount of time. We have also had challenges in using the IMARIS software to automate the hair cell quantification. So far all tests with IMARIS have produced far more hair cell counts than what we achieve by counting manually. We are working with the IMARIS technical support team to resolve this problem.

Specific Aim 1C: *In vitro* drug screen using cochlear explants from *Prox1*^{CreERT2}*::Rosa26*$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$ mice that have fate-mapping of supporting cells and hair cell damage induced by neomycin.

Combining neomycin-induced hair cell damage with fate-mapping of supporting cells will allow discrimination of *de novo* hair cell regeneration from hair cell protection or repair. Here we created cochlear explants from *Prox1*^{CreERT2}*::Rosa26* $^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$ pups which express tdTomato in the supporting cells that surround outer hair cells after tamoxifen injection (given at P0). Similar to Aim 1B, explants were created at P2 and treated with neomycin for 24 hours to induce hair cell damage. After washes in media, the explants were treated with the candidate compounds that passed Aim 1B for 96 hours and fixed tissue was immunostained with the hair cell marker, myosin VIIA. Using confocal microscopy, we examined the entire middle and basal turns of the cochlea to

identify any Tomato+ hair cells. We also quantified the number of inner and outer hair cells in the middle and basal turns of the cochlea similar to Aim 1B.

In year 1, we have tested five compounds in Aim 1C (1-3 doses per compound) (Table 2). Two compounds were advanced from Aim 1A and the other three were identified by Otonomy prior to the start of this award using cochlear explants from wild-type animals. Preliminary data from Oto-74 shows no change in hair cell numbers in explants treated with the compound (Figure 3A), but we have a small sample size for two of the doses tested. Analysis of Tomato+ hair cells in these samples is underway. At the 10µM dose, Oto-38 did not show an increase in the number of hair cells compared to neomycin-treated controls (Figure 3B) and also did not produce any Tomato+ hair cells. However there were many ectopic hair cells in the Oto-38 samples located lateral to the organ of Corti (Figure 3C,D) which suggests that supporting cell subtypes differ in their response to inhibition of the Rho-kinase pathway. We plan to increase the sample size for the 100uM dose of Oto-38. We have a small sample size at 1 or 2 doses for Oto-4, Oto-13, and Oto-79 and the analysis is in progress.

Table 2: Compounds tested in Aim 1C

Compound	Mechanism of Action	Doses tested	Outcome
Oto-4	DNMT inhibitor	30µM	Analysis in progress
Oto-13	DNMT inhibitor	10µM, 100µM	Analysis in progress
Oto-38	Rho-kinase inhibitor	10µM, 100µM	No Tomato+ HCs, extra HCs lateral to OoC
Oto-74	Lats kinase inhibitor	3µM, 10µM, 30µM	Analysis in progress
Oto-79	microRNA	30µM, 100µM	Analysis in progress

Abbreviations: DNA methyltransferase (DNMT), Hair cells (HCs), organ of Corti (OoC).

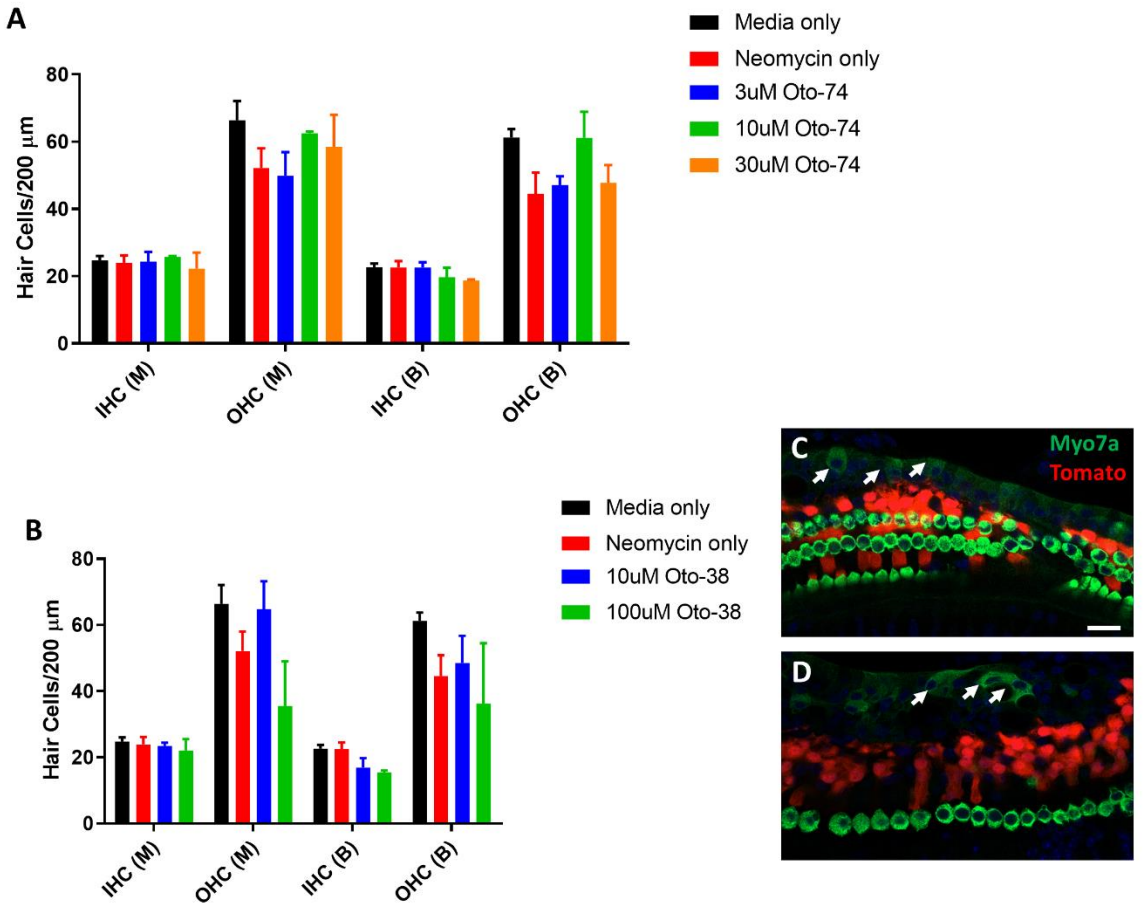


Figure 3: Quantification of hair cells from cochlear explants created from *Prox1*^{CreERT2}*::Rosa26*$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$ mice that have fate-mapping of supporting cells and hair cell damage induced by neomycin. **A,** There was no significant difference in the number of hair cells found in explants treated with Oto-74 [3µM n=7 (blue bars); 10µM n=3 (green bars); 30µM n=2 (orange bars) compared to neomycin-treated controls (red bars, n=11). **B,** There was no significant difference in the number of hair cells found in explant treated with Oto-38 [10µM n=5 (blue bars); 100µM n=2 (green bars)] compared to neomycin-treated controls (red bars, n=11). Black bars in A, B were treated with media only (n=7). **C, D,** Representative confocal images of cochlear explants treated with neomycin followed by 10µM Oto-38. Hair cells are labeled by myosin VIIa (Myo7a, green). Ectopic hair cells are present lateral to the organ of Corti (arrows) in both images. Scale bar = 20µm.

Stated goals not met: Aim 1C is behind schedule. We are again performing all analyses at SIUSOM which is taking an increased amount of time.

Specific Aim 2: *In vivo* drug screen using fate-mapping and noise exposure.

The project is designed such that all Aims are rolling and overlapping. As soon as a compound passes one subAim, it will move to the next step. None of the compounds we have tested so far in Aim 1C have produced enough regenerated hair cells to advance to Aim 2.

Stated goals not met: Aim 2 is delayed since none of the compounds we have tested so far have produced enough regenerated hair cells to warrant the *in vivo* testing. Because of this delay, we have not completed the back-crossing of the *Fgfr3-iCre^{ERT2}:: Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice planned in Aim 2B.

What opportunities for training and professional development has the project provided?

Training
Melanie Bedolli, and Becky Rose (Technicians) were trained by Dr. Cox, Jarnail Singh and Debbie Hamilton to learn all the techniques needed for the project such as mouse breeding, genotyping, creation of cochlear explants, immunostaining, confocal microscopy, and image quantification. Sujata Pandey (graduate student) and Hannah Johnson (medical student) were trained by Dr. Cox, Jarnail Singh, Melanie Bedolli, and Debbie Hamilton on these same procedures.

Professional Development
Dr. Cox attended the Association for Research in Otolaryngology 46th annual midwinter meeting held 11-15-FEB-2023 in Orlando, FL.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Our goals for the next reporting period are:
1) Aim 1A: Test Oto-24 at 10µM
2) Aim 1A: Increase the sample size for the 3 concentrations of Oto-69.
3) Aim 1A: Test additional doses of Oto-10 (lower than 1µM)
4) Aim 1A: Test additional compounds from the library with doses on a log scale.
5) Aim 1B: Continue testing Oto-39 at other doses
6) Aim 1B: Test Oto-33 at 5µM and 500nM
7) Aim 1C: Increase the sample size for 2 concentrations of Oto-74 & complete the analysis of Tomato+ hair cells.
Aim 1C: Continue testing Oto-38 at 100µM
8) Aim 1C: Continue analysis and testing of Oto-4, Oto-13, and Oto-7
9) Aim 1C: Test Oto-49 at 5µM and 50µM
11) Aim 2A: Back-cross *Fgfr3-iCre^{ERT2}:: Rosa26$^{loxP\text{-}stop\text{-}loxP\text{-}tdTomato}$* mice to CBA/CaJ mice

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

In September 2023, Otonomy downsized their research group and in December 2023 the company folded. Thus Co-PI Bonnie Jacques and Co-I Phillip Uribe are no longer involved in the project. As described in the grant application, Otonomy provided the compound library and the *in vitro* drug screening in Aim 1 will all occur in the Cox lab at SIUSOM. However all of the Aim 1 analysis is now being done in the Cox lab (instead of jointly). Otonomy's main role was in Aims 2A and 2C which we plan to achieve by partnering with a chemist for the formulation work and a CRO for the pharmacokinetic work (Aim 2A). Aim 2C can be conducted in the Cox lab and we will learn the intra-tympanic injection method from another expert. We have initiated conversations with a chemist at SIU Edwardsville who Dr. Cox has worked with previously and with a local CRO who has expertise in inner ear drug delivery and pharmacokinetics. We informed program officer Jami Scheib of these changes via email on 7-Sept-2023 and had virtual meetings on 9-Sept-2023 and 14-Dec-2023 to discuss the details.

Actual or anticipated problems or delays and actions or plans to resolve them

Due to Otonomy's closure all of the Aim 1 analysis is now being done in the Cox lab (instead of jointly) which has slowed progress. In addition, we have had challenges in using the IMARIS software to automate the hair cell quantification. So far all tests with IMARIS have produced far more hair cell counts than what we achieve by counting manually. We are working with the IMARIS technical support team to resolve this problem.

Changes that had a significant impact on expenditures

In regards to the budget, Otonomy's contributions were all cost-shared. We now need to purchase the compounds instead of them being provided by Otonomy which will increase our commodities budget. We will also need funds to support the collaboration with the chemist and CRO for Aim 2A and for the experiments planned for Aim 2C. The additional consequence of Otonomy's closure is that Dr. Helen Valentine was not able to train with them on the intra-tympanic injection method. Thus she has not contributed to the project in year 1 and her budgeted salary support has not been used. Once we have identified a compound that meets the criteria for the *in vivo* testing in Aim 2 we will re-budget. Unless additional funds can be obtained, these changes will likely result in a decreased number of compounds that we can screen.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects or vertebrate animals

No deviations have occurred. All work has been conducted for Aim 1 only following approved protocols. We will ensure ACURO approval of the CRO IACUC protocol for Aim 2A and the addition of Aim 2C to the SIUSOM IACUC protocol before any animal work is done on those Aims.

No human subjects were used in research supported by this award.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Brandon C. Cox, PhD**
Project Role:	PI
Researcher Identifier:	ORCID 0000-0002-6989-161X
Nearest person month worked:	3
Contribution to Project:	Responsible for all aspects of the research conducted under this award including hiring and training personnel, experimental design, data analysis and interpretation, administrative duties, managing the budget, and reporting to the DOD
Funding Support:	NIH R01 AG073151, NIH R01 DC00151, NIH R01 DC13771, NIH R01 MH129749, W81XWH-19-1-0017 & Decibel Therapeutics, Inc.
Name:	**Melanie Bedolli**
Project Role:	Researcher I (Technician)
Nearest person month worked:	10
Contribution to Project:	Trained personnel, managed mouse colony, created cochlear explants & treated them with test compounds, performed immunostaining, administrative duties, and ordered supplies
Funding Support:	NIH R01 AG073151
Name:	**Becky Rose**
Project Role:	Researcher I (Technician)
Nearest person month worked:	7
Contribution to Project:	Assisted with mouse colony maintenance, performed confocal imaging, and quantified confocal images
Funding Support:	NIH R01 AG07315 & NIH R01 DC13771
Name:	**Debbie Hamilton**
Project Role:	Researcher III (Technician)
Nearest person month worked:	4
Contribution to Project:	Trained personnel, assisted in mouse colony maintenance, quantified confocal images, administrative duties, and ordered supplies
Funding Support:	NIH R01 AG073151

Name:	**Jarnail Singh, PhD**
Project Role:	Staff Scientist
Researcher Identifier:	ORCID 0000-0001-6032-2376
Nearest person month worked:	7
Contribution to Project:	Trained personnel, project management, created cochlear explants & treated them with test compounds, and data analysis
Funding Support:	NIH R01 AG073151

Name:	**Sujata Pandey**
Project Role:	Graduate Student
Nearest person month worked:	6
Contribution to Project:	Assisted with mouse colony maintenance, created cochlear explants & treated them with test compounds, performed immunostaining & confocal imaging, and quantified confocal images
Funding Support:	None

Name:	**Hannah Johnson**
Project Role:	Medical Student
Nearest person month worked:	2
Contribution to Project:	Performed confocal imaging and quantified confocal images
Funding Support:	None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Melanie Bedolli and Becky Rose are the 2 TBD Researchers who were hired once this grant was awarded. Otonomy, Inc folded in December 2023 and thus Co-PI Bonnie Jacques and Co-I Phillip Uribe are no longer involved in the project; however their salaries were cost-shared. In addition this prevented Dr. Helen Valentine from training with Otonomy on the intra-tympanic injection method. Since we have not started Aim 2 yet, Dr. Valentine has not contributed to the project in year 1.

What other organizations were involved as partners?

1) Otonomy, Inc, San Diego, CA, in-kind support: supplied the AI-generated compound library
2) St. Jude Children's Research Hospital, Memphis, TN, in-kind support: supplied Prox1-CreER mice

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Development of New Therapies that Stimulate Hair Cell Regeneration

RH210028, W81XWH-22-1-0674

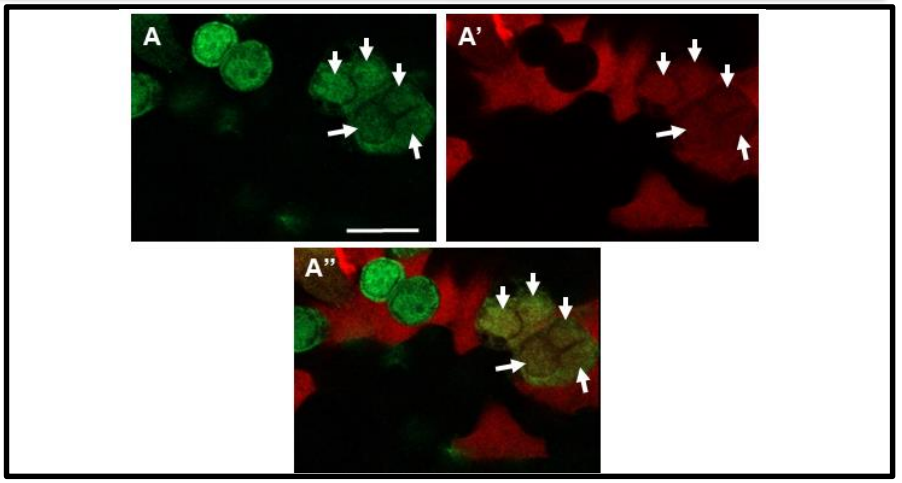
PI: Brandon Cox, PhD **Org:** Southern Illinois Univ. School of Medicine **Award Amount:** $1,475,000

Study/Product Aim(s)

Aim 1: *In vitro* drug screen using cochlear explants
Aim 2: *In vivo* drug screen using fate-mapping and noise exposure

Approach

Proposed studies will use an improved, more definitive, drug screening method to evaluate an artificial intelligence generated library of potentially regenerative compounds in hopes of identifying compounds that stimulate cochlear hair cell regeneration. Positive hits in the *in vitro* explant screens will be formulated for local otic delivery, and undergo pharmacokinetic and safety/toxicity profiling followed by *in vivo* efficacy studies in an adult model of noise-induced hearing loss. Both *in vitro* and *in vivo* screening will employ fate-mapping of supporting cells to confirm that compounds act through a regenerative mechanism.



Fate-mapping allows discrimination of hair cell (HC) regeneration versus HC protection by labeling any newly formed HCs that were derived from supporting cells. Here tdTomato+ HCs (arrows) confirms that the candidate compound (OMY-750) stimulates regeneration in a cochlear explant model.

Timeline and Cost

Activities / CY	22-23	23-24	24-25
Aim 1A & 1B screening			
Aim 1C screening			
Aim 2A formulation & screening			
Aim 2B & 2C screening			
Estimated Budget ($K)	**$505**	**$480**	**$489**

Updated: (8/24/2023)

Goals/Milestones

CY22-23 Goals

☑ Begin screening compounds in naïve explant model (1A) & explant model with hair cell damage (1B)
☑ Begin screening compounds in explant model with fate-mapping and hair cell damage (1C)

CY23-24 Goals

☐ Begin formulation work on compounds that pass Aim 1 (2A)
☐ Begin pharmacokinetic and safety/toxicity profiling (2A)
☐ Begin *in vivo* efficacy studies using model with noise induced hearing loss (2B & 2C)
☐ Continue *in vitro* screening compounds in Aim 1 using explants

CY24-25 Goals

☐ Continue screening compounds in both Aims

Budget Expenditure to Date

Actual Expenditure: $379,667 for Y1