Durable Recovery of Auditory Function Following Intracochlear Delivery of AK-OTOF (AAVAnc80-hOTOF Vector) in a Translationally Relevant Mouse Model of Otoferlin Gene (OTOF)-mediated Hearing Loss

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Disclosures

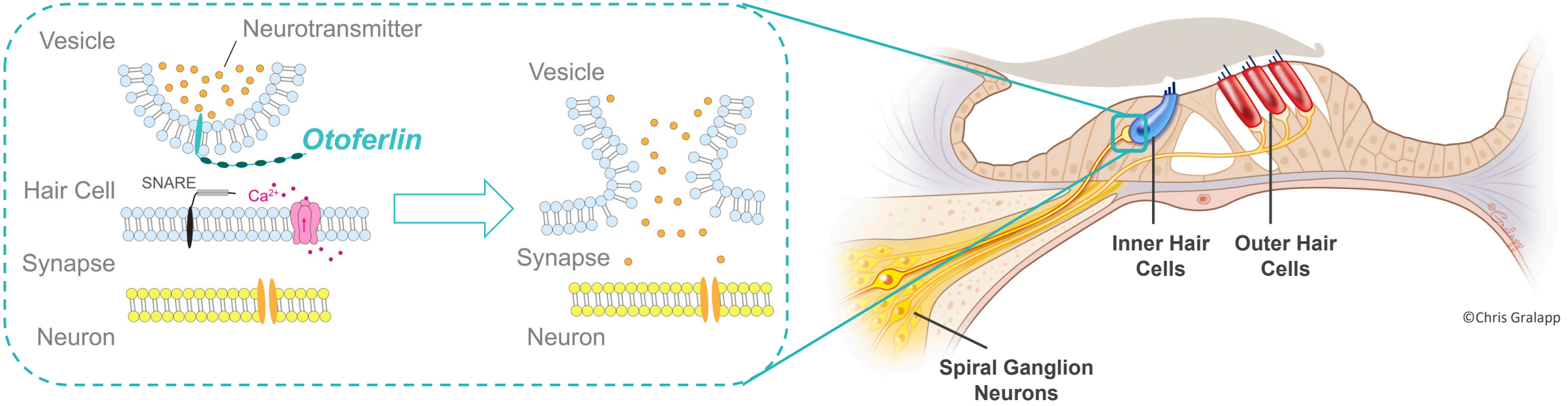
Ann Hickox is an employee of Akouos, Inc., and has received, and is receiving, compensation and equity from Akouos, Inc.

Forward Looking Statements

This presentation includes “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995, including, but not limited to: the ability of our product candidate AK-OTOF to potentially restore, improve, and preserve high-acuity physiologic hearing; statements relating to the initiation, plans, and timing of our future clinical trials and our research and development programs; the timing of our planned IND submission for AK-OTOF; and the geographic expansion of the Resonate™ program. These forward-looking statements may be accompanied by such words as “aim,” “anticipate,” “believe,” “continue,” “could,” “estimate,” “expect,” “forecast,” “goal,” “intend,” “may,” “might,” “plan,” “potential,” “possible,” “target,” “will,” “would,” and other words and terms of similar meaning. Akouos may not actually achieve the plans, intentions, or expectations disclosed in these forward-looking statements, and you should not place undue reliance on these forward-looking statements. Actual results or events could differ materially from the plans, intentions, and expectations disclosed in these forward-looking statements as a result of various factors, including: our limited operating history; uncertainties inherent in the development of product candidates, including the initiation and completion of nonclinical studies and clinical trials; the timing of and our ability to submit and obtain regulatory approval; whether results from nonclinical studies will be predictive of results or success of clinical trials; our ability to obtain sufficient cash resources to fund our foreseeable and unforeseeable operating expenses and capital expenditure requirements; our ability to obtain, maintain, and enforce our intellectual property; the impact of the COVID-19 pandemic on our business, results of operations, and financial condition; the potential that our internal manufacturing capabilities and/or external manufacturing supply may experience delays; risks related to competitive programs; and the other risks and uncertainties that are described in the Risk Factors section of the Company's Annual Report on Form 10-K for the year ended December 31, 2020, which is on file with the Securities and Exchange Commission, and in other filings that Akouos may make with the U.S. Securities and Exchange Commission. These statements are based on our current beliefs and expectations as of the date of this presentation. We do not undertake any obligation to publicly update any forward-looking statements except as required by law. By attending or receiving this presentation, you acknowledge that: you are cautioned not to place undue reliance on these forward-looking statements; you will be solely responsible for your own assessment of the market and our market position; and you will conduct your own analysis and be solely responsible for forming your own view of the potential future performance of Akouos, Inc.

Otoferlin: an Essential Protein for Hearing

- The *OTOF* gene encodes otoferlin, a protein that plays a critical role in the priming, fusion, and replenishing of synaptic vesicles at the inner hair cell (IHC) synapse during sound encoding
- The lack of normal otoferlin protein in the cochlea impairs synaptic signaling between the cells that sense sound energy (IHCs) and the cochlear nerve fibers (*i.e.,* spiral ganglion neurons) that transmit sound information to the brain



- As a consequence of impaired synaptic signaling, the highly synchronized neuronal responses required for hearing are diminished in individuals with mutations in *OTOF*

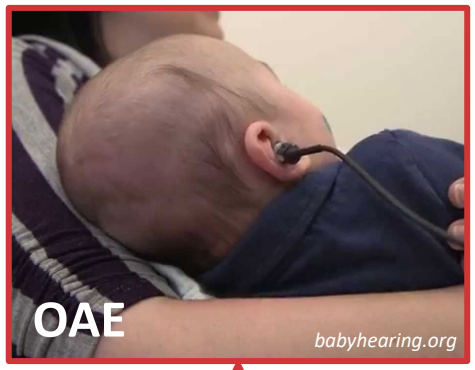
(Yasunaga 1999 Nat Genet; Pangršič 2012 Trends Neurosci)

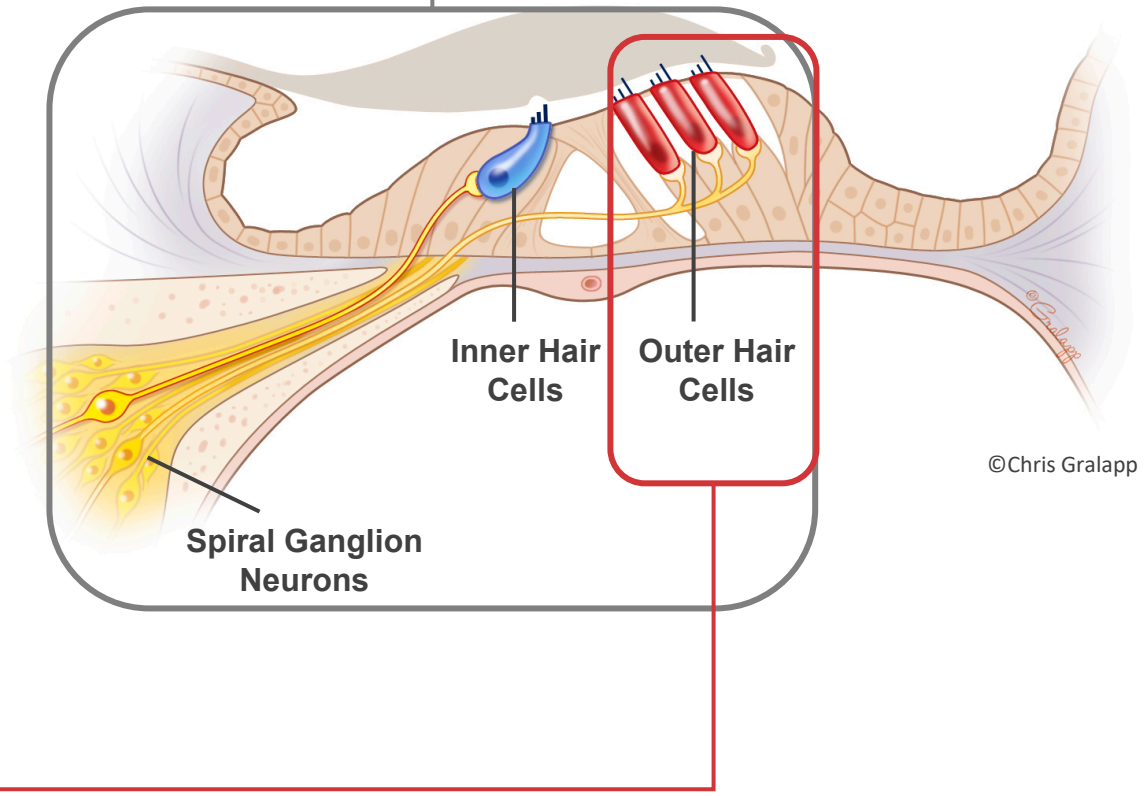
Identification of *OTOF*-mediated Hearing Loss

- Individuals with biallelic mutations in the *OTOF* gene typically have a congenital, Severe to Profound sensorineural hearing loss (*OTOF*-mediated hearing loss)
- *OTOF* gene mutations can be suspected when the auditory brainstem response (ABR) is absent and otoacoustic emissions (OAEs) are present; genetic testing confirms *OTOF*-mediated hearing loss

- Absent / abnormal ABR reflects impaired neural transmission of sound information from ear to brain, which can delay or prevent spoken language acquisition

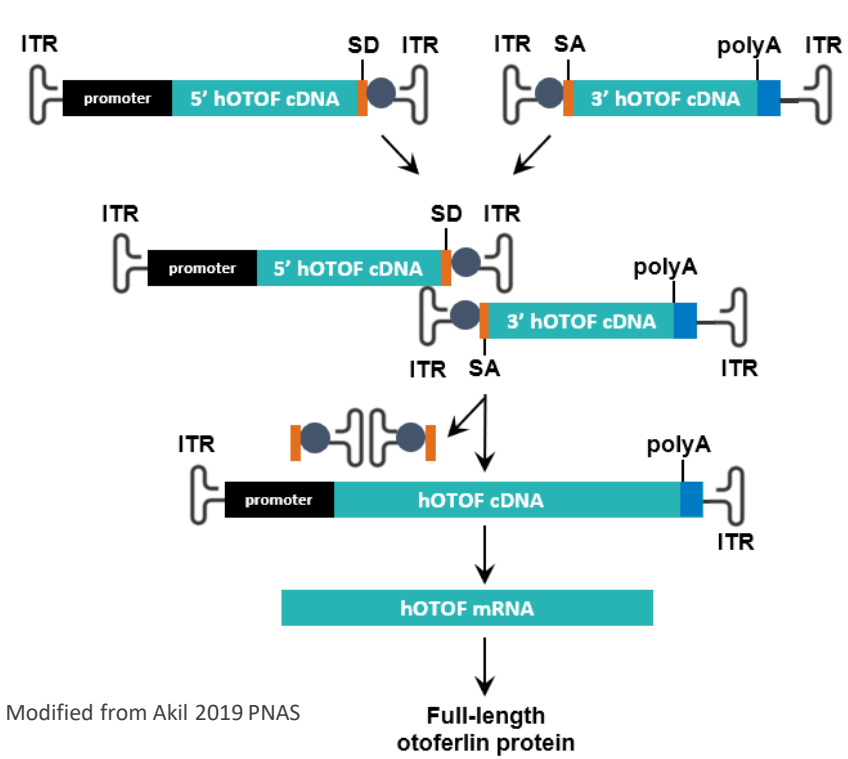
- Present OAEs reflect outer hair cell activity and cochlear integrity (*i.e.*, with the exception of inner hair cell signaling to neurons, the cochlea responds normally to sound)





Development of AK-OTOF for *OTOF*-mediated Hearing Loss

- AK-OTOF, a product candidate in preclinical development, is a dual AAVAnc80 vector encoding the nearly 6 kB cDNA human otoferlin under the control of a ubiquitous promoter; it is intended to treat individuals with *OTOF*-mediated hearing loss by gene transfer and durable expression of a normal, functional otoferlin protein following intracochlear administration



Modified from Akil 2019 PNAS

Transduction

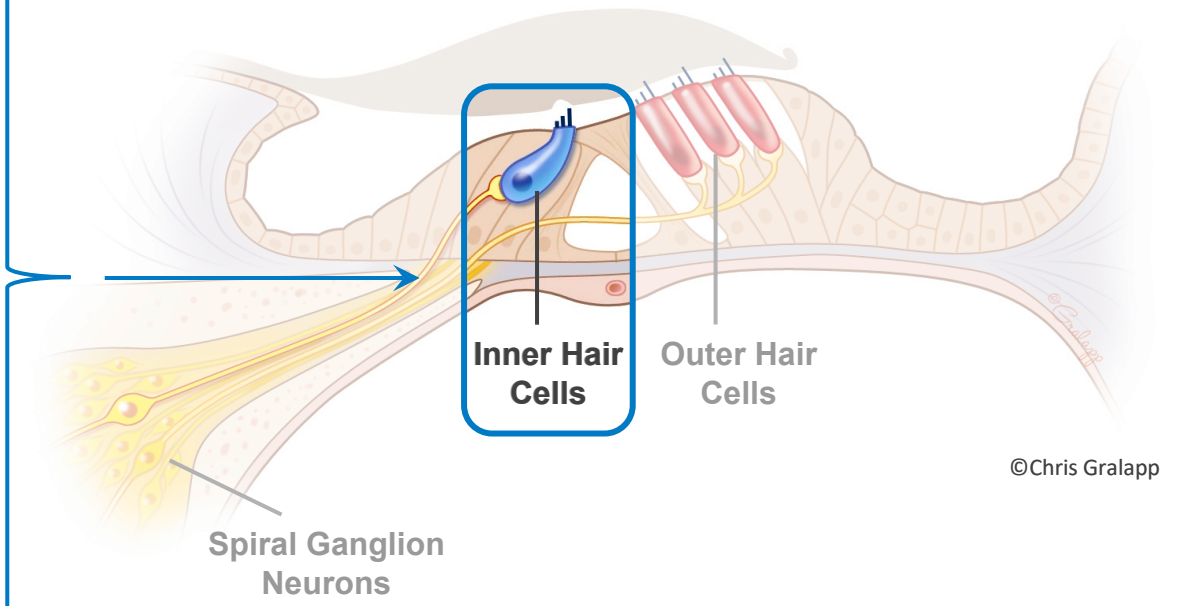
DNA sequence alignment

Recombination

Transcription

Splicing

Translation



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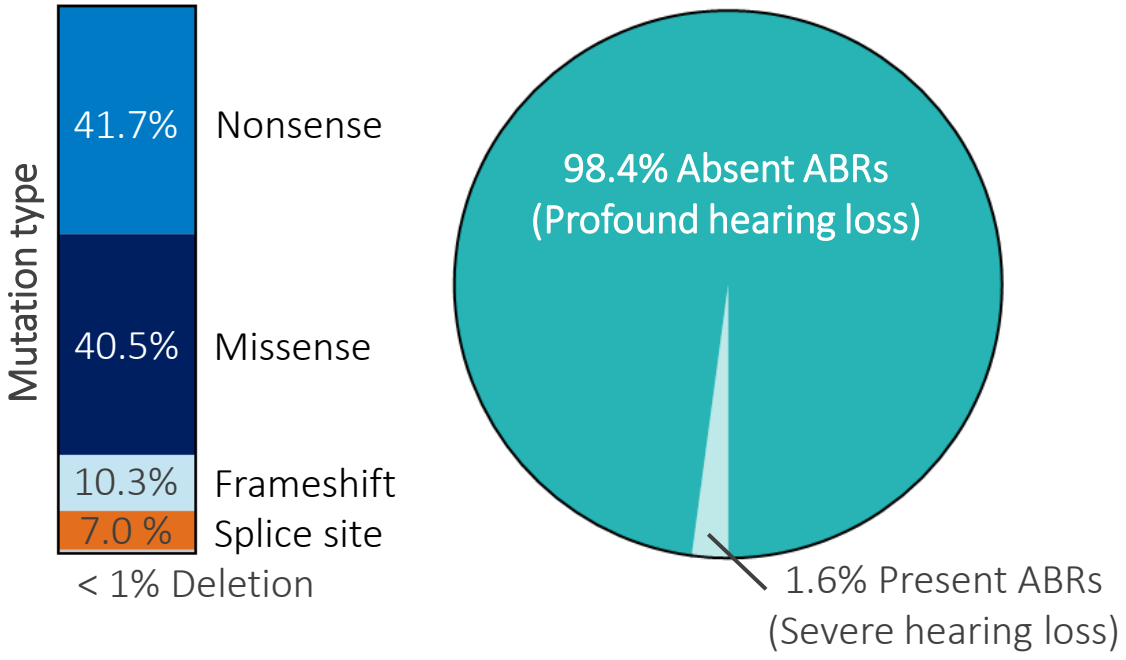
- *Gene therapy for OTOF-mediated hearing loss is expected to confer the greatest benefit when global cochlear function is normal,* i.e.*, synaptic signaling between the IHCs and SGNs (also referred to as cochlear nerve fibers) is the primary deficit*

IHCs: inner hair cells; SGNs: spiral ganglion neurons

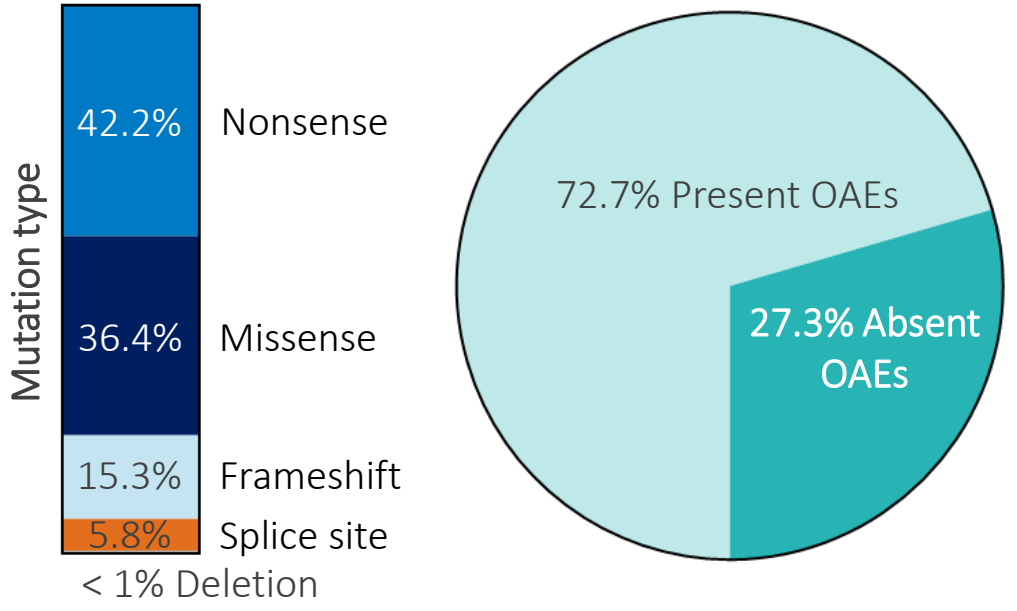
Natural History of *OTOF*-mediated Hearing Loss

- Comprehensive analysis of 130 peer-reviewed articles yielded genetic and audiologic data from 533 individuals with biallelic mutations in the *OTOF* gene with a stable (*i.e.*, not temperature-sensitive) phenotype
- ABR and OAE data were analyzed for individuals with pathogenic or likely pathogenic mutations:

As expected, auditory brainstem response (ABR) was widely absent, across a spectrum of mutation types (n = 121 individuals; n = 242 alleles)



However, otoacoustic emission (OAE) presence was inconsistent (n = 154 individuals; n = 308 alleles) in this primarily young (< 11 years) population, where age available

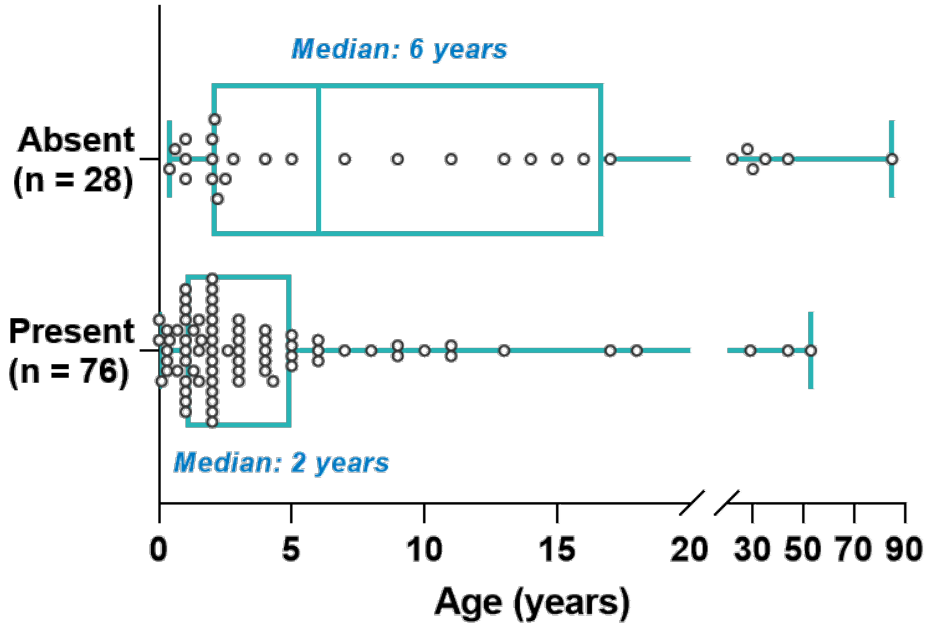


Pathogenicity determined by ACMG criteria plus available clinical data
Present ABR: reported present or detected at 90 dB HL (Severe hearing loss);
Absent ABR: reported absent or detected at > 90 dB HL (Profound hearing loss)

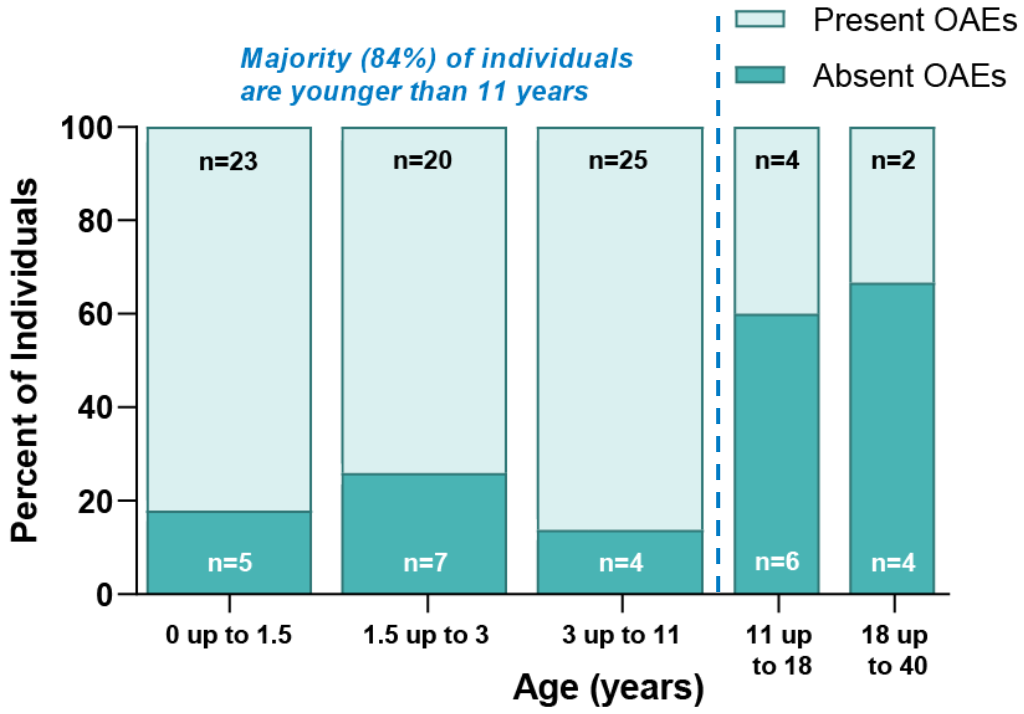
OAEs: DPOAEs (distortion product OAEs) or TEOAEs (transient evoked OAEs), reported as present or absent

OAEs Decline with Age in *OTOF*-mediated Hearing Loss

- OAE responses are reported to diminish or disappear within the first decade of life in individuals with *OTOF*-mediated hearing loss (Rodriguez-Ballesteros 2003 Hum Mutat; Kitao 2019 Ear Hear)
- In the comprehensive literature review, the majority of OAE data were from individuals under 11 years, and median age was greater for those with absent OAEs (n = 104 with age reported at test)



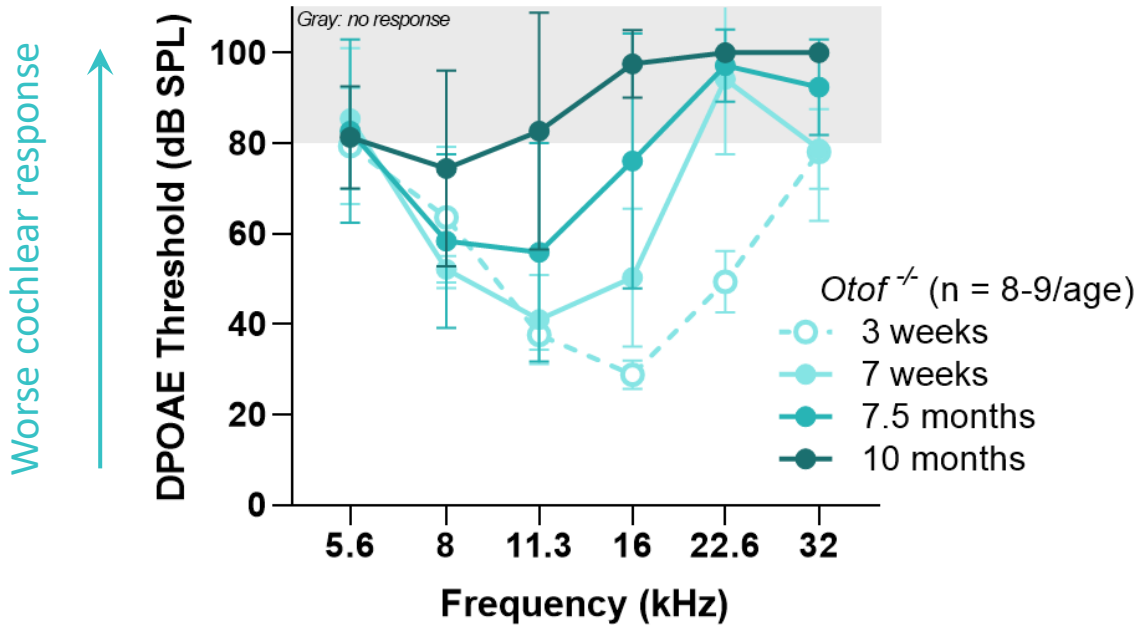
- Based on the comprehensive literature review data, OAEs can be absent within the first few years of life, and the likelihood of identifying individuals with present OAEs substantially decreases within the first decade
- Individuals with present OAEs may receive the most potential benefit from AK-OTOF; present OAEs are more likely in younger individuals



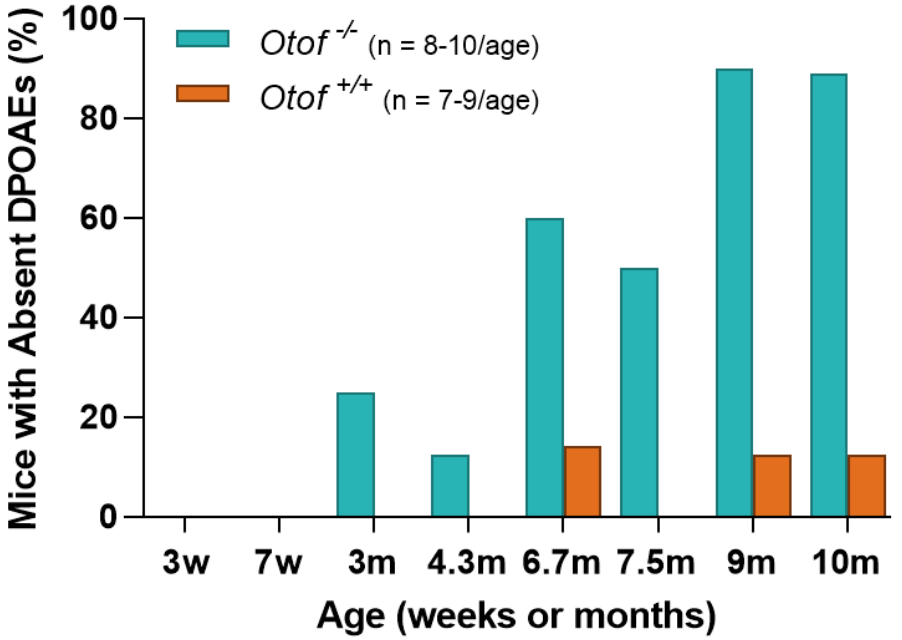
Individuals with pathogenic / likely pathogenic, biallelic mutations in the *OTOF* gene with a stable (*i.e.*, not temperature-sensitive) phenotype; present if OAEs present in at least one ear; oldest age used if longitudinal data available

OAEs Decline with Age in an *Otof* Knockout Mouse Model

- ABRs are absent (no ABR threshold up to 105 dB SPL; data not shown) in the *Otof* $^{-/-}$ mouse at all ages tested (from 3 weeks to 10 months of age)
- Paralleling data from the comprehensive literature review in humans with *OTOF*-mediated hearing loss, distortion product OAE (DPOAE) thresholds increase (*i.e.*, responses decline) with age in the knockout (*Otof* $^{-/-}$) mouse



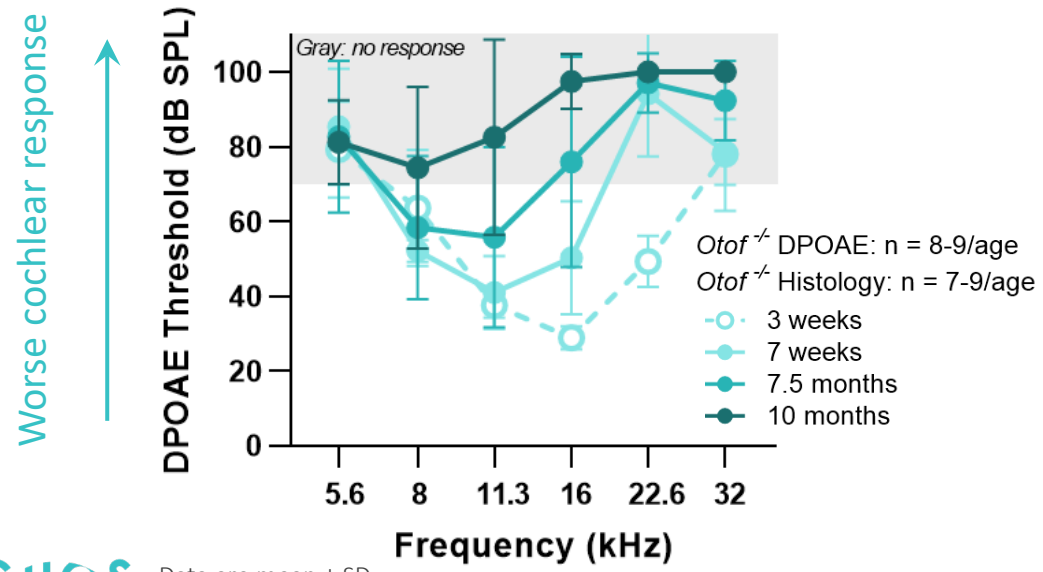
- DPOAE absence increases more rapidly with age in the knockout (*Otof* $^{-/-}$) mouse compared to the wild-type (*Otof* $^{+/+}$) mouse, indicating an effect of genotype and not simply an effect of mouse strain
  - Absent = No DPOAE response in 50% or more of the 4 test frequencies (8, 11.3, 16, 22.6 kHz) where a change in response can be observed

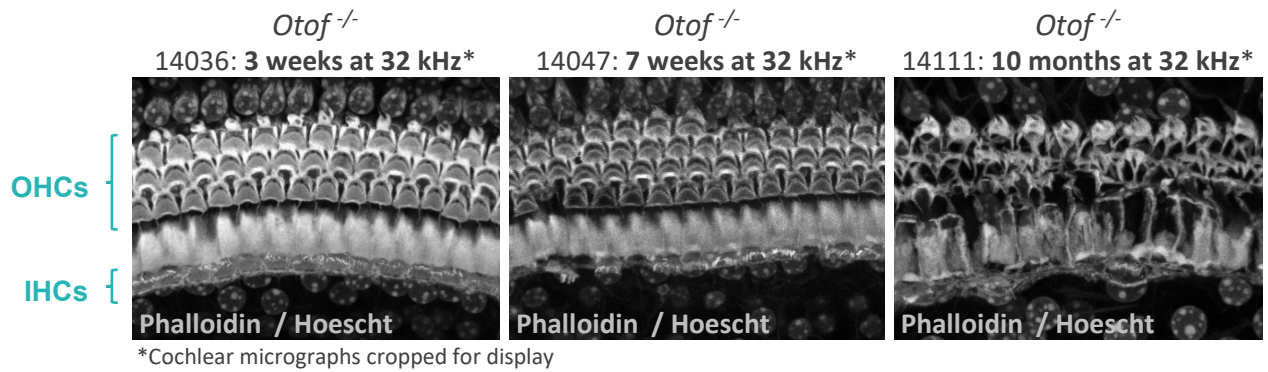


Data are mean ± SD
If no DPOAE response was detected up to L1 / L2 = 90 / 80 dB SPL (highest presentation level), threshold was imputed at 100 dB SPL and included in mean

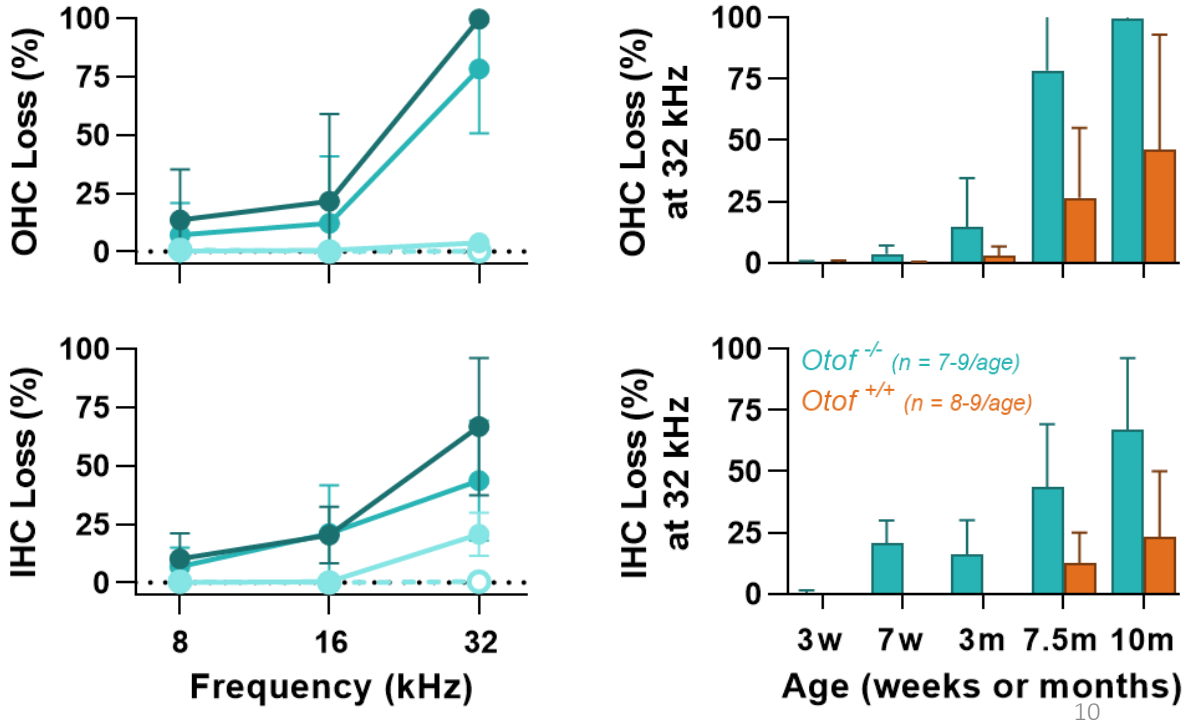
OAE Decline as a Biomarker for Cochlear Dysfunction

- DPOAE decline is seen at 7 weeks of age in the *Otof* $^{-/-}$ mouse model despite minimal-to-no outer hair cell (OHC) loss, indicating dysfunction of an origin other than loss of the cells that generate the response. Inner hair cell (IHC) loss appears as early as 7 weeks of age, further suggesting degenerative processes within the cochlea
- Both OHC and IHC loss increase more rapidly with age in the knockout (*Otof* $^{-/-}$) mouse compared to the wild-type (*Otof* $^{+/+}$) mouse





*Cochlear micrographs cropped for display

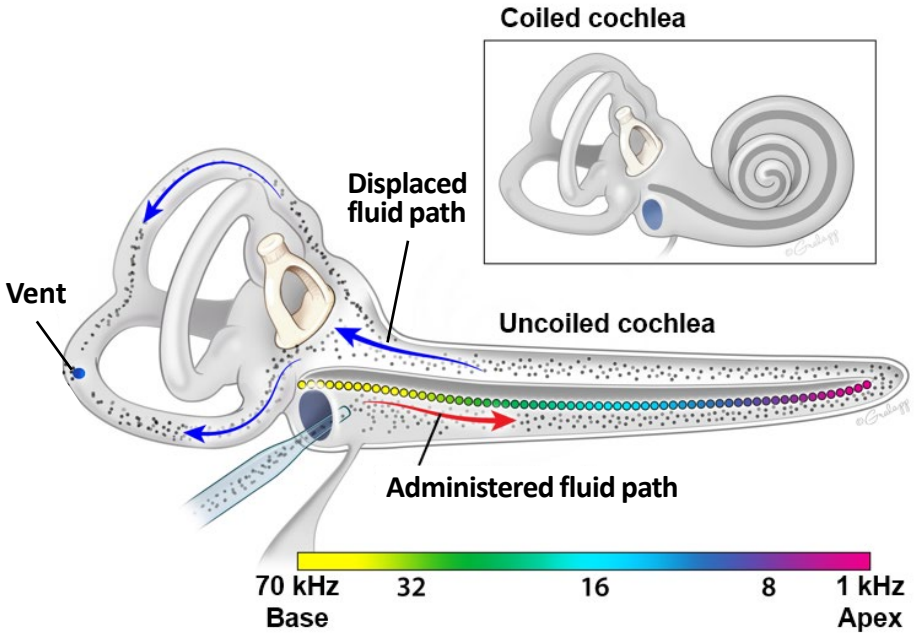


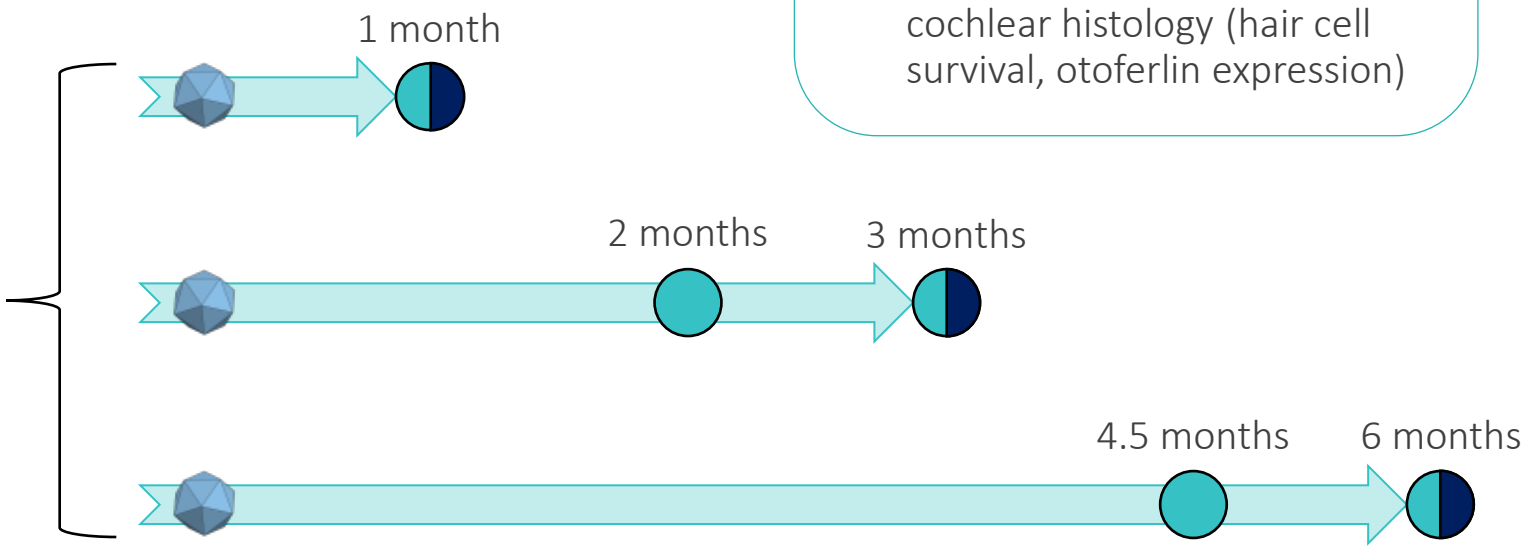
Data are mean ± SD

If no DPOAE response was detected up to L1 / L2 = 90 / 80 dB SPL (highest presentation level), threshold was imputed at 100 dB SPL and included in mean

Administration of AK-OTOF Prior to OAE Decline

- Durability of functional recovery was evaluated following intracochlear administration of AK-OTOF at an age when the cochlea is mature but prior to the decline of DPOAEs (*i.e.*, decline in cochlear integrity)
- AK-OTOF or vehicle was administered to P23 ± 2 knockout (*Otof* $^{-/-}$) mice, and vehicle was administered to P23 ± 2 wild-type (*Otof* $^{+/+}$) mice
- The dual vectors comprising AK-OTOF were administered at a 1:1 ratio (5':3'), which gives optimal mRNA and protein expression *in vitro* (ASGCT 2021 abstract #355)





Intracochlear administration of AK-OTOF or vehicle control

Cochlear and auditory function tests (DPOAE / ABR)

Cochlear and auditory function tests (DPOAE / ABR) and cochlear histology (hair cell survival, otoferlin expression)

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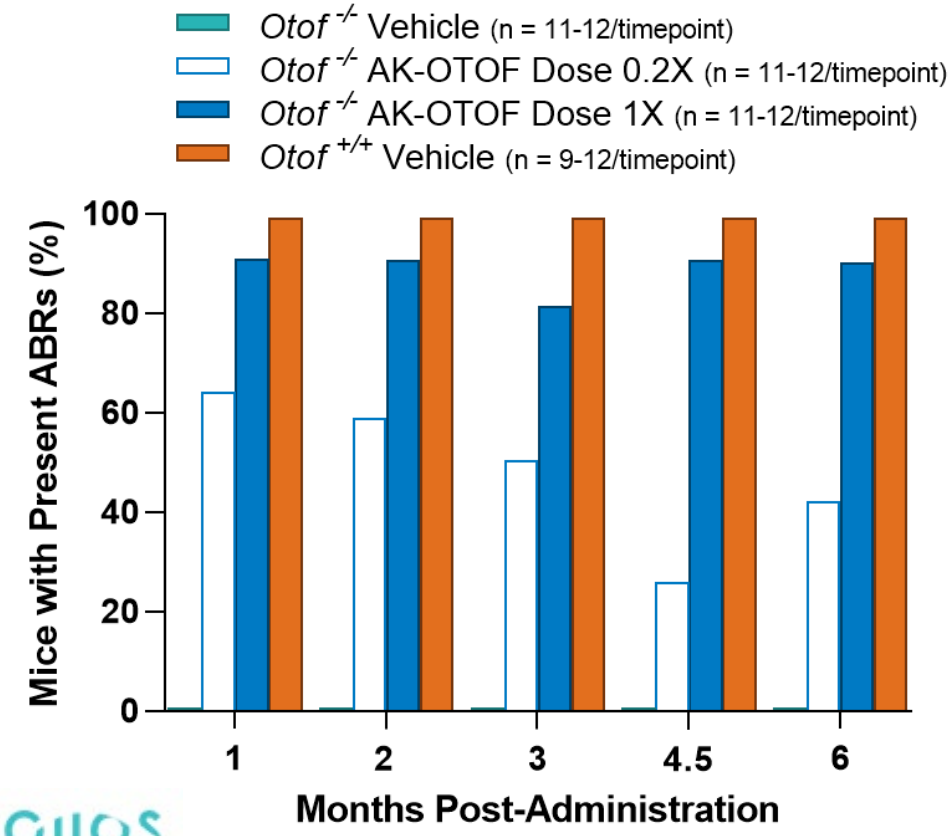
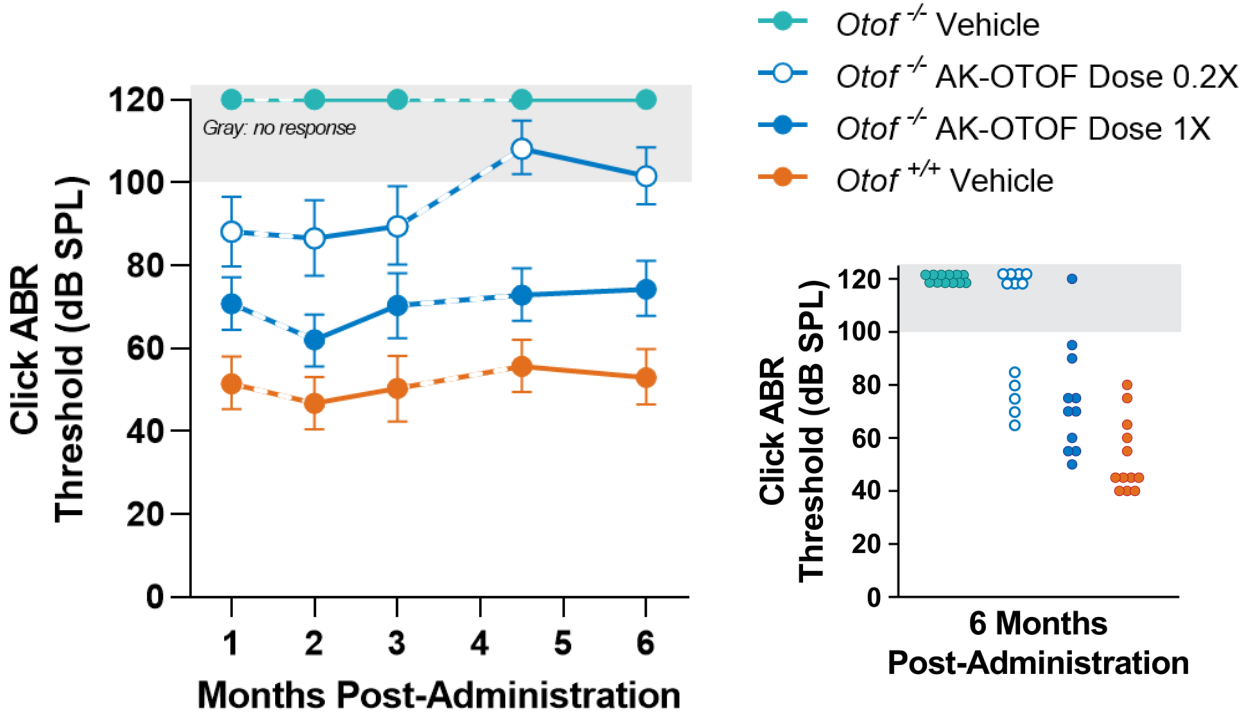
Intracochlear Administration of AK-OTOF to *Otof* Knockout Mice Resulted in Durable Restoration of Auditory Function

- Unlike *Otof* $^{-/-}$ mice administered vehicle, which have no measurable auditory brainstem responses (ABRs), at least 80% of *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 1X had detectable ABRs throughout the full study duration



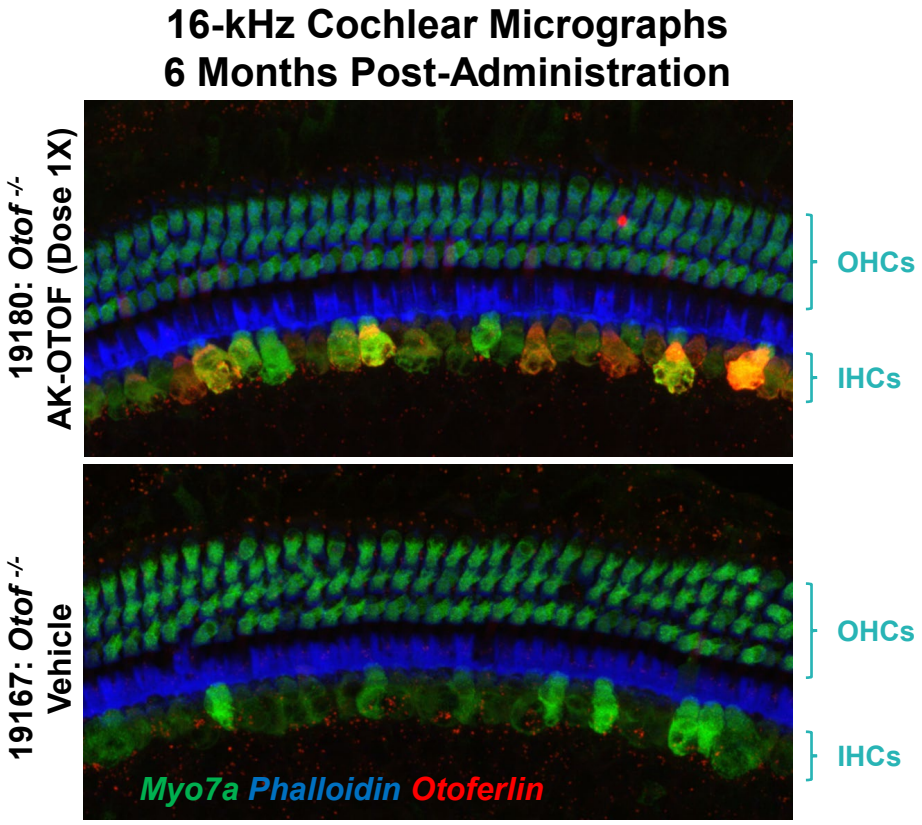
- ABRs indicate functional restoration to near-WT (*Otof* $^{+/+}$) thresholds in *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 1X
- The extent of auditory function restoration was dependent on the dose administered



Data (above, left) are mean ± SEM; dashed lines reflect a separation of cohorts according to the study design, whereas solid lines indicate data are from the same animals.
Data (above, right) represent individual animals.
If no ABR was detected up to 105 dB SPL (highest presentation level), threshold was imputed at 120 dB SPL and included in mean

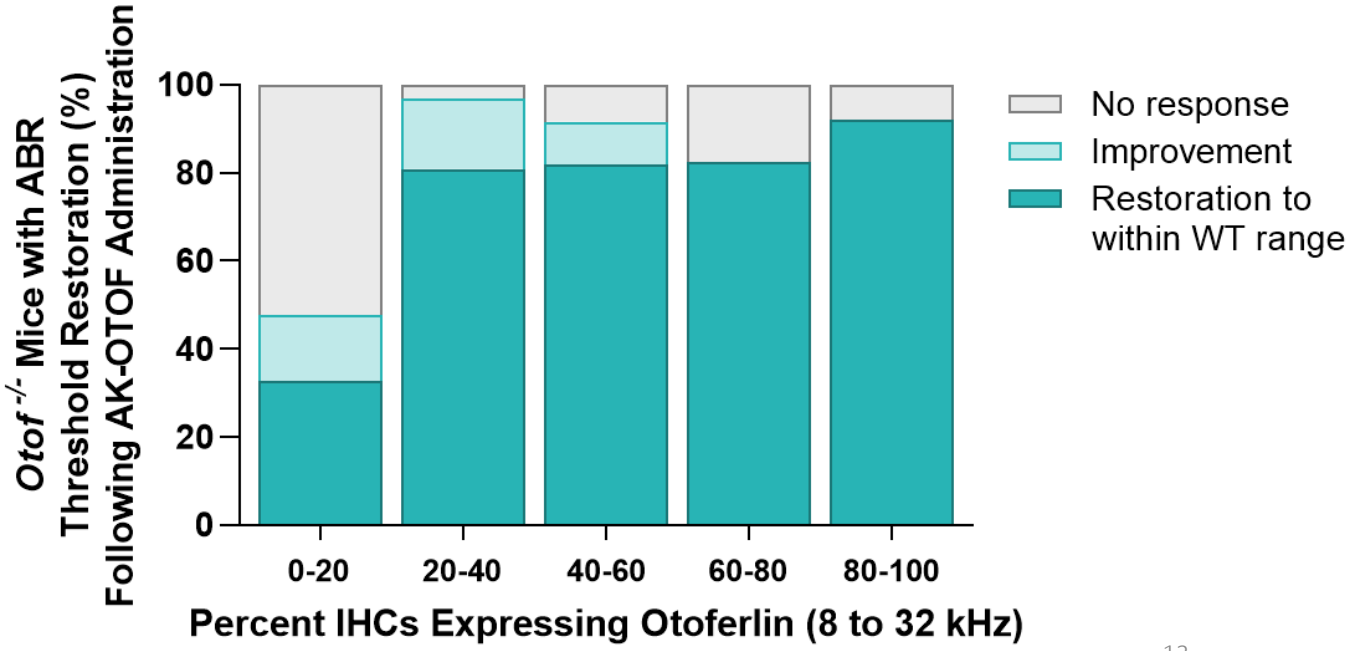
AK-OTOF Administration to *Otof* Knockout Mice Resulted in Durable Expression of Human Otoferlin Sufficient for Sustained Restoration of Auditory Function

- *Otof* $^{-/-}$ mice showed durable expression of human otoferlin protein in inner hair cells (IHCs) that was detectable by immunohistochemistry 6 months post-administration of AK-OTOF



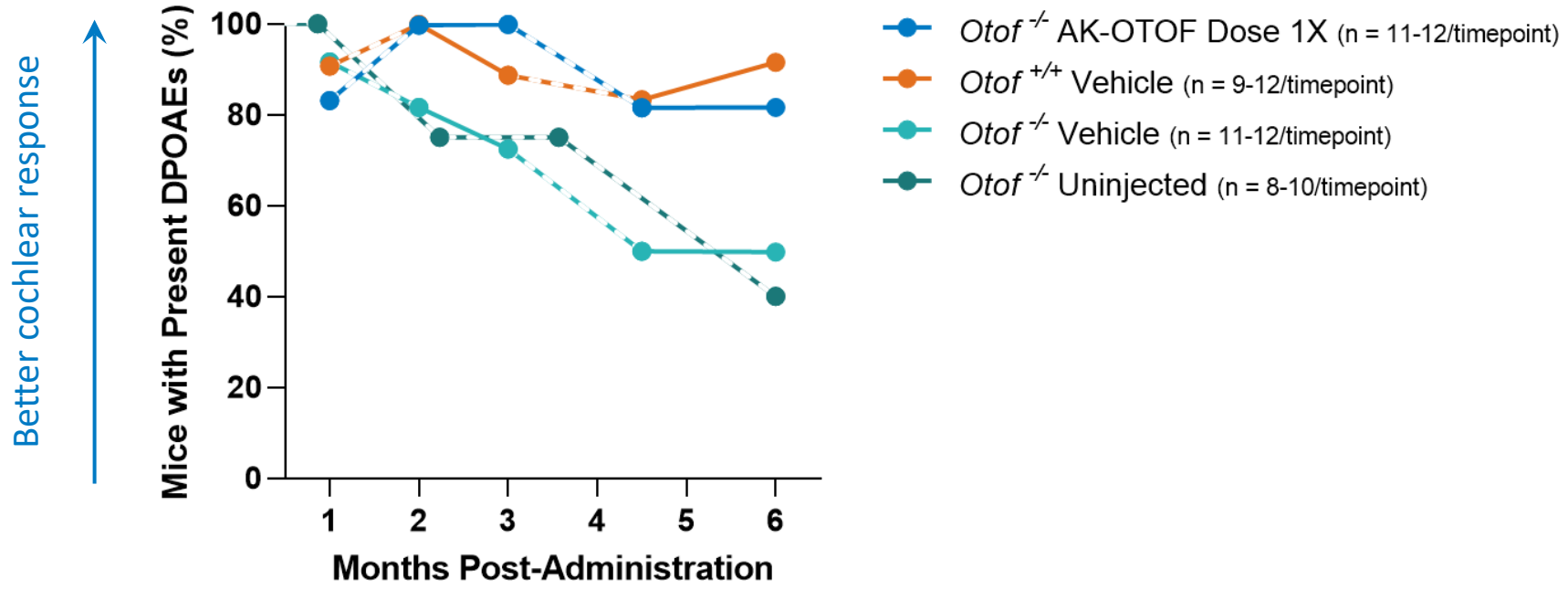
Cochlear micrographs represent maximum projections through confocal image stacks; OHC = outer hair cells

- Expression of human otoferlin in 20% or more of IHCs results in restoration of auditory function (click ABR thresholds) to within the range of wild-type (*Otof* $^{+/+}$, WT) mice
  - Click-ABR thresholds at 1, 3, and 6 months were pooled across all doses (n = 106 *Otof* $^{-/-}$ mice)
  - The percentage of *Otof* $^{-/-}$ mouse IHCs expressing otoferlin following administration of AK-OTOF was computed from confocal image stacks (like those on the left), averaged across 8, 16, and 32 kHz frequency positions, and pooled across 1, 3, and 6 months and across all doses



Intracochlear Administration of AK-OTOF May Preserve OAEs in the *Otof* Knockout Mouse Model

- Following administration of vehicle, DPOAEs remained present in most wild-type (*Otof* $^{+/+}$) mice over 6 months but were increasingly absent in *Otof* $^{-/-}$ mice, paralleling the rate of DPOAE absence over time in the uninjected *Otof* $^{-/-}$ mice
- In *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 1X, the rate of DPOAE presence was 80% or higher, similar to *Otof* $^{+/+}$ mice administered vehicle, suggesting that early administration of AK-OTOF prior to decline of OAEs has the potential to preserve OAE responses; OAEs reflect cochlear function and cochlear integrity



Of the 3 DPOAE frequencies assessed (8, 16, 32 kHz), 16 kHz data were analyzed for absence/presence
16-kHz DPOAEs were "present" if a response was measurable at L1 / L2 = 85 / 75 dB SPL or below
Dashed lines reflect a separation of cohorts according to the study design, whereas solid lines indicate data are from the same animals;
Uninjected *Otof* $^{-/-}$ data are from the characterization of the mouse model represented on pages 9 and 10 (same animals)

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Summary

- AK-OTOF is a dual AAVAnc80 vector in preclinical development and is intended to treat individuals with *OTOF*-mediated hearing loss by delivering the human *OTOF* gene to inner hair cells

- Individuals with *OTOF*-mediated hearing loss have impaired neural transmission of sound (absent / abnormal auditory brainstem response [ABR]) and are likely to experience a decline in cochlear integrity typically within the first decade of life, indicated by initially present, then absent, otoacoustic emissions (OAEs)

- An *Otof* knockout mouse model recapitulates the decline in OAEs over time that is observed in reports of individuals with *OTOF*-mediated hearing loss, demonstrating the biological relevancy of this mouse model to support the planned clinical development of AK-OTOF and indicating the possibility of cochlear sensory cell loss with age

- Administration of AK-OTOF to an *Otof* knockout mouse model prior to OAE decline can result in durable restoration of auditory function, as measured by ABRs, and may preserve OAEs

- Taken together, these findings support the planned clinical development of AK-OTOF for the treatment of hearing loss in individuals with confirmed biallelic *OTOF* mutations, as identified through genetic testing

Genetic Testing for Eligible Individuals with Auditory Neuropathy

resonate™ BY AKOUOS

Akouos is partnering with Blueprint Genetics to provide access to a potential genetic diagnosis at no cost to eligible individuals, their insurance, or their healthcare providers

- Today, few individuals with auditory neuropathy receive a genetic diagnosis. A key barrier is the availability and accessibility of genetic testing
- A genetic diagnosis for auditory neuropathy:
  - Can empower individuals to make informed choices, foster connections with others living with disabling hearing loss, and may provide valuable insight into medical management
  - Could help individuals and their healthcare providers determine potential eligibility for future clinical trials
- To be eligible for the program, individuals:
  - Can be any age;
  - Must have a current or prior clinical diagnosis of bilateral auditory neuropathy, or a medical history consistent with bilateral auditory neuropathy; and
  - Must not have a syndromic medical history
- Participants in the program have access to the Blueprint Genetics Comprehensive Hearing Loss and Deafness Panel that includes more than 230 genes associated with genetic forms of hearing loss

The program is available in the United States and plans to expand to additional geographic regions throughout 2021


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