Preclinical Development of a Genetic Medicine for Otoferlin Gene-mediated Hearing Loss: AK-OTOF

AKOUOS

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Disclosures

Yuan Gao is an employee of Akouos, Inc., a wholly owned subsidiary of Eli Lilly and Company, and has received and is receiving compensation from Akouos, Inc.

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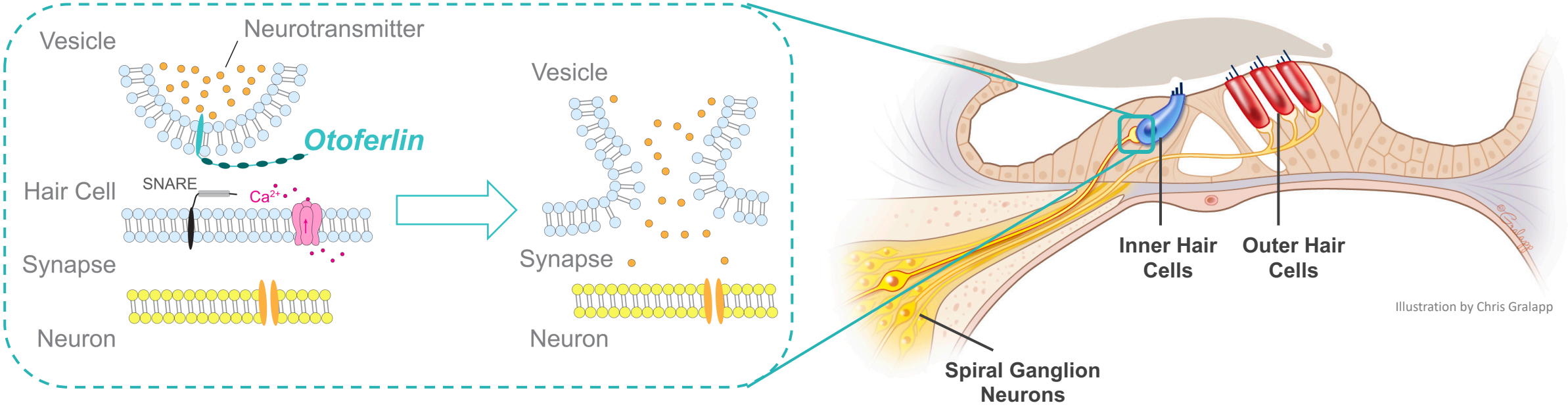
Otoferlin: an Essential Protein for Hearing

- The otoferlin gene (*OTOF*) encodes otoferlin, a protein that plays a critical role in the priming, fusion, and replenishing of synaptic vesicles at the IHC synapse during sound encoding
- The lack of normal otoferlin protein impairs synaptic signaling, typically resulting in bilateral, Severe to Profound sensorineural hearing loss



Abbreviations: IHC = inner hair cell; *OTOF* = otoferlin gene.
References: Modified from Yasunaga 1999, Pangršič 2012.

Preclinical Development of AK-OTOF for Potential Treatment of *OTOF*-mediated Hearing Loss

Development of the approach to deliver genetic medicines to target cells in the inner ear:

- Design and delivery parameters of AK-OTOF
- Design of the Akouos delivery device and an intracochlear administration procedure that translates from animal models to humans

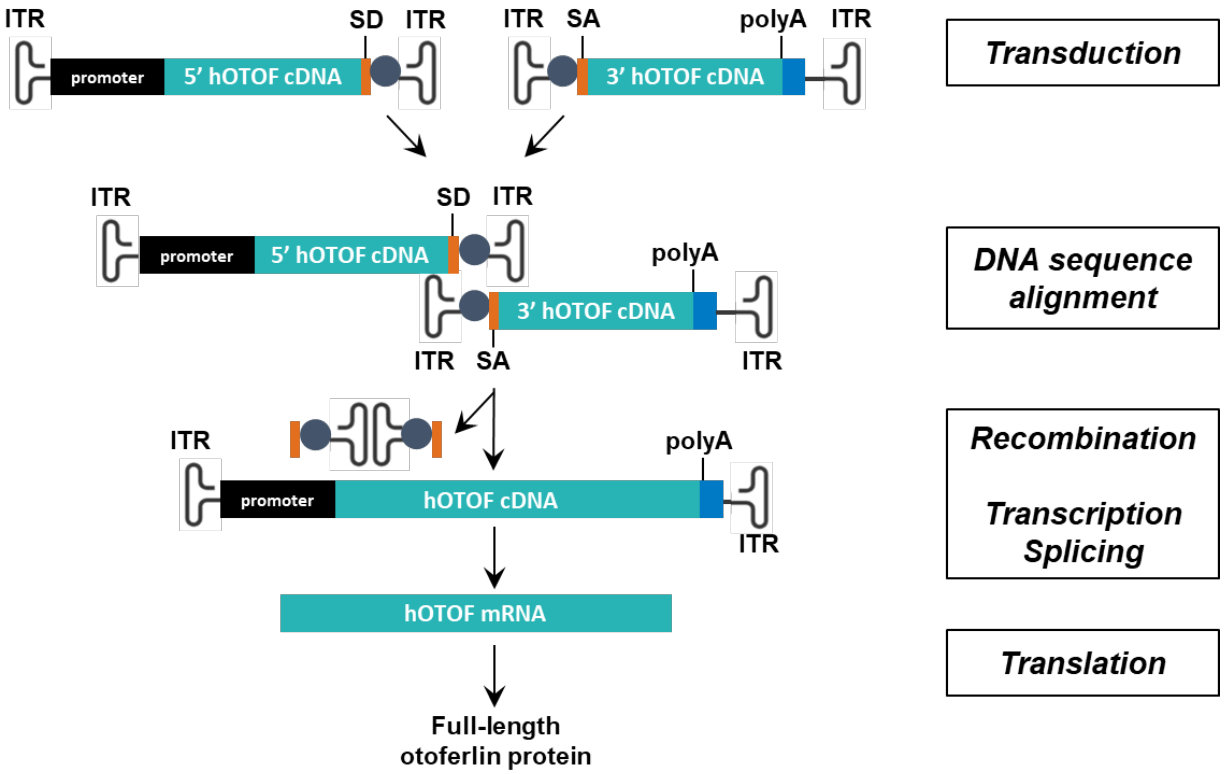
Nonclinical studies inform the design of clinical studies:

- Demonstrate relevance of the disease model to the human population
- Demonstrate biological plausibility by the intended clinical route of administration
- Identify biologically active dose levels, and the onset and durability of functional recovery
- Evaluation of intervention window (with respect to OAE status)
- Evaluation of safety of AK-OTOF

Abbreviation: OAE = otoacoustic emission.

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

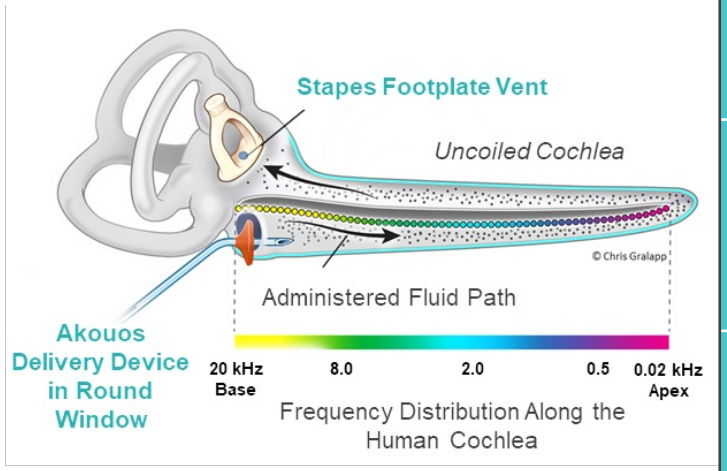
- AK-OTOF utilizes a dual vector approach: two component AAVAnc80 vectors (5’ and 3’) together encode the approximately 6 kB human otoferlin cDNA under the control of a ubiquitous promoter

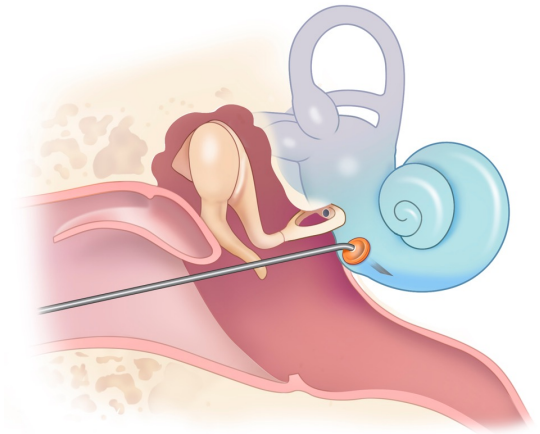


Reference: modified from Akil 2019.

Abbreviations: AAVAnc80 = adeno-associated viral vector Anc80 variant; cDNA = complementary DNA; hOTOF= human otoferlin; ITR = inverted terminal repeats; kB = kilobases; mRNA = messenger RNA; *OTOF* = human otoferlin gene; polyA = polyadenylation tail; SA = splice acceptor; SD = splice donor.

Intracochlear Delivery is Similar Across Mammalian Species





Key Attributes of Delivery Approach	Humans	NHPs	Mice
Intracochlear Administration via RWM	✓	✓	✓
Fenestration	✓ Stapes footplate	✓ Stapes footplate	✓ PSCC
Akouos's Delivery Device	✓	✓ (modified for NHP)	✗ Glass micropipette
Minimally Invasive Surgical Approach	✓ Transcanal Tympanotomy (through EAC)	✗ Post-auricular Transmastoid / Facial Recess (drilling into mastoid bone)	✗ Post-auricular (drilling into otic bulla)

Abbreviations: EAC = external auditory canal; NHP(s) = non-human primate(s); PSCC = posterior semicircular canal; RWM = round window membrane.

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- Demonstrate relevance of the disease model to the human population
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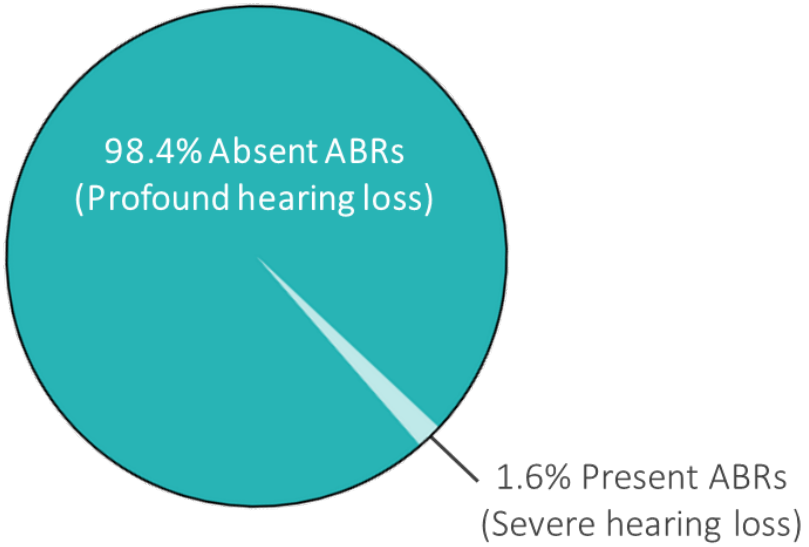
- Evaluation of safety of AK-OTOF

Abbreviation: OAE = otoacoustic emission.

Natural History of *OTOF*-mediated Hearing Loss

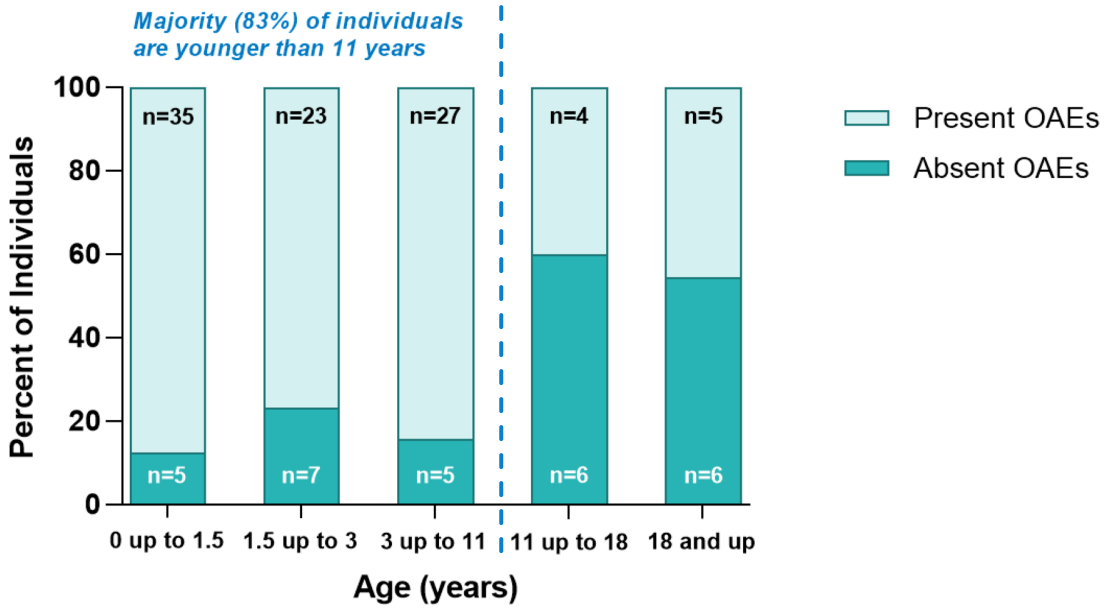
ABRs were absent in nearly all individuals



n = 121 individuals
Absent ABR: reported absent or detected at > 90 dB HL (Profound hearing loss)
Present ABR: reported present or detected at 90 dB HL (Severe hearing loss)

OAE presence was inconsistent in this primarily young population

The likelihood of identifying individuals with present OAEs substantially decreases within the first decade



OAEs: DPOAEs (distortion product OAEs) or TEOAEs (transient evoked OAEs), reported as present or absent
n = 123 individuals with OAE status and age at testing reported

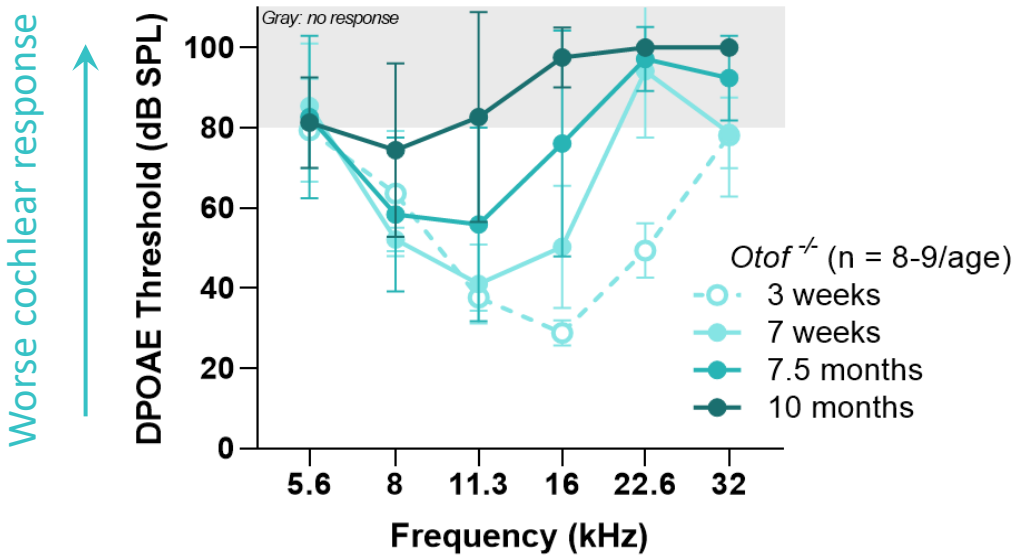
Comprehensive analysis of 130 peer-reviewed articles yielded genetic and audiologic data from 533 individuals with biallelic *OTOF* mutations and a stable (*i.e.*, not temperature-sensitive) phenotype
ABR and OAE data were analyzed for individuals with pathogenic or likely pathogenic mutations, determined by ACMG criteria plus available clinical data

Abbreviations: ABR(s) = auditory brainstem response(s); ACMG = American College of Medical Genetics and Genomics; dB HL = decibels hearing level; OAE(s) = otoacoustic emission(s); *OTOF* = human otoferlin gene.

The *Otof* $^{-/-}$ Mouse Model Recapitulates the Phenotype of *OTOF*-mediated Hearing Loss

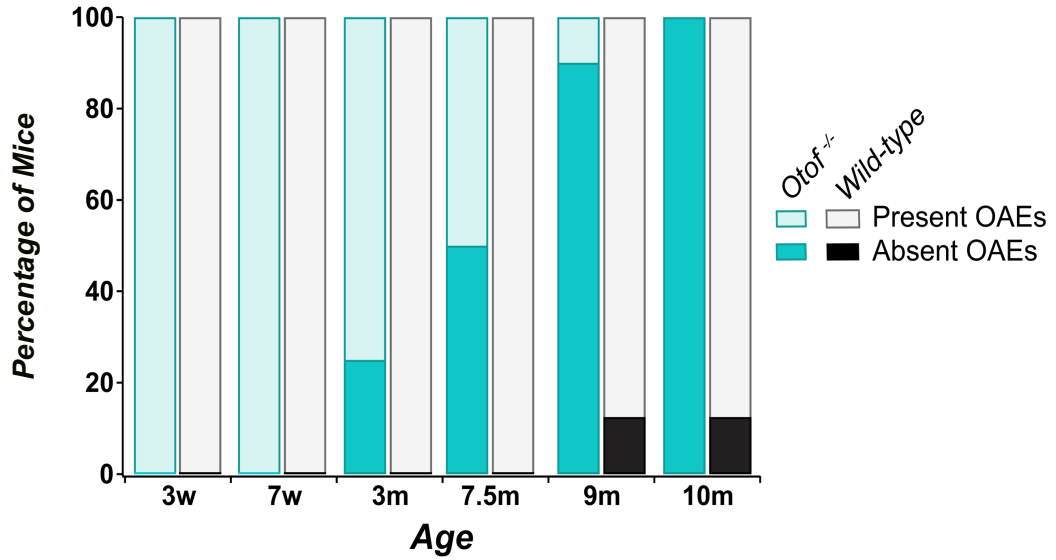
ABRs are absent in the *Otof* $^{-/-}$ mouse at all ages tested (from 3 weeks to 10 months of age; data not shown)

DPOAE thresholds increase as early as 7 weeks and continue to worsen with age



Study AK-014: Data are mean ± SD; If no DPOAE response was detected up to L_1 / L_2 = 90 / 80 dB SPL (highest presentation level), threshold was imputed at 100 dB SPL and included in mean
Absent ABR = no response up to 105 dB SPL

DPOAE absence progresses more rapidly with age in *Otof* $^{-/-}$ mice compared to WT mice, indicating an effect of genotype and not simply an effect of mouse strain



Study AK-014 in *Otof* $^{-/-}$ mice (n = 8 to 10 / age) and WT mice (n = 7 to 9 / age)
Absent DPOAE = No response in up to L_1 / L_2 = 90 / 80 dB SPL for 50% or more of the 4 test frequencies (8, 11.3, 16, 22.6 kHz) where a change in response can be observed

Abbreviations: ABRs = auditory brainstem responses; dB SPL = decibels sound pressure level; DPOAE(s) = distortion product otoacoustic emission(s); kHz = kilohertz; m = months; OAEs = otoacoustic emissions; *Otof* $^{-/-}$ = otoferlin knock-out; SD = standard deviation; w = weeks; WT = wild-type.

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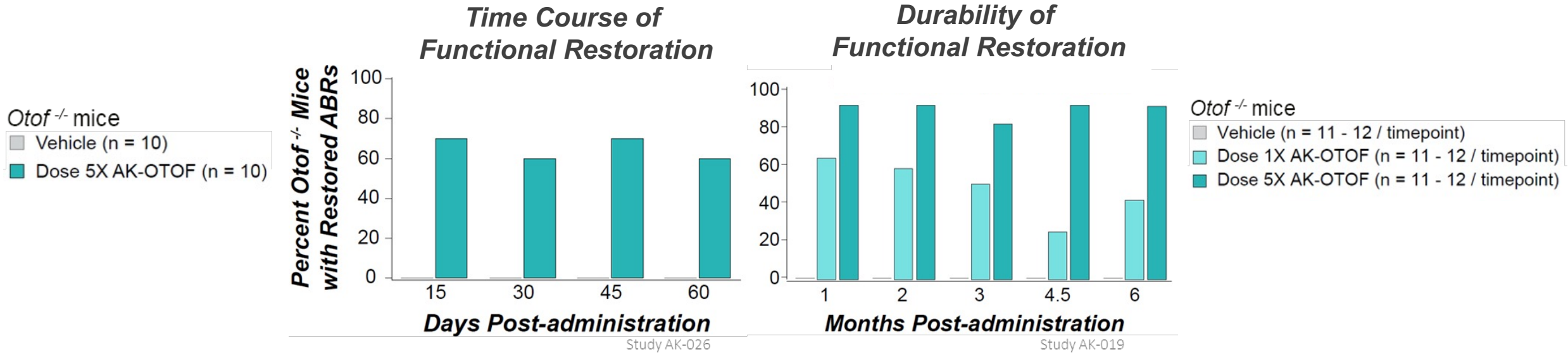
AK-OTOF Restores Auditory Function When Delivered to Juvenile *Otof* $^{-/-}$ Mice with Mature Cochleae

- Intracochlear administration of AK-OTOF to 3-week-old *Otof* $^{-/-}$ mice leads to expression of human otoferlin protein expression in IHCs and restores auditory function (ABR) by Day 15 and through ≥ 6 months (the longest survival duration evaluated)
- The extent of ABR restoration (degree of response and responder rate) was dependent on dose administered

~ 70% of *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 5X showed restored ABRs by Day 15

≥ 80% of *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 5X showed restored ABRs through ≥ 6 months

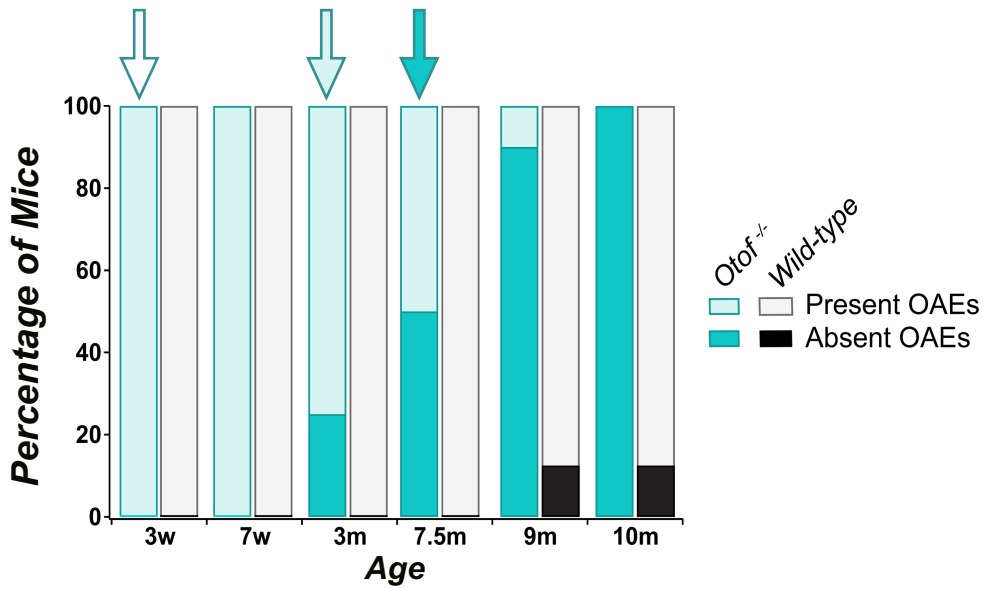


Restored ABR = Click-evoked ABR threshold within the range of vehicle-injected WT mice

Abbreviations: ABR(s) = auditory brainstem response(s); IHCs = inner hair cells; *Otof* $^{-/-}$ = otoferlin knock-out.

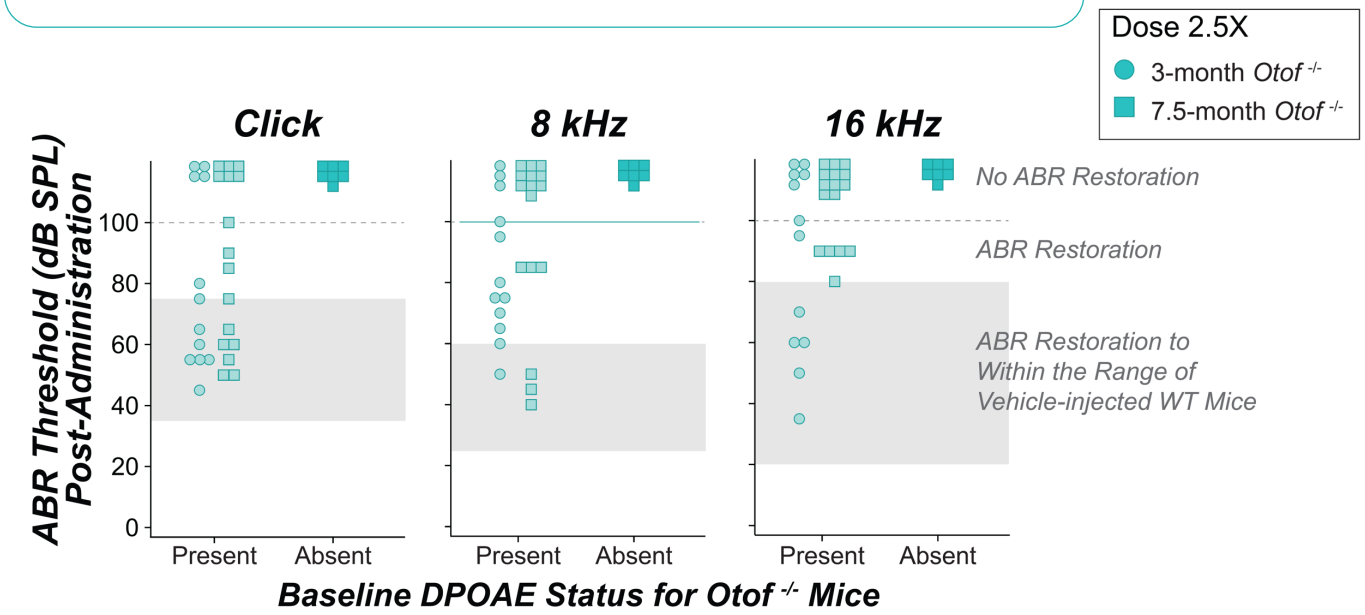
Restoration of Auditory Function is Less Likely Following OAE Decline

The intervention window for otoferlin-mediated hearing loss was evaluated in *Otof* $^{-/-}$ mice aged to 3 or 7.5 months



Study AK-014 in *Otof* $^{-/-}$ mice (n = 8 to 10 / age) and WT mice (n = 7 to 9 / age)
Absent DPOAE = No response up to L_1 / L_2 = 90 / 80 dB SPL for 50% or more of four test frequencies (8, 11.3, 16, 22.6 kHz)

AK-OTOF can restore auditory function in older mice, provided DPOAEs are present at the time of administration



Study AK-015 in *Otof* $^{-/-}$ mice at 3 months (n = 12) and 7.5 months (n = 23) of age
Absent DPOAE = No response up to L_1 / L_2 = 90 / 80 dB SPL for 50% or more of four test frequencies (8, 11.3, 16, 22.6 kHz)

These nonclinical data suggest that humans with present OAEs may receive the most potential benefit from AK-OTOF; present OAEs are more likely in younger individuals.

Abbreviations: ABR(s) = auditory brainstem response(s); dB SPL = decibels sound pressure level; DPOAE(s) = distortion product otoacoustic emission(s); kHz = kilohertz; m = months; OAE(s) = otoacoustic emission(s); OHC = outer hair cell; *Otof* $^{-/-}$ = otoferlin knock-out; w = weeks; WT = wild-type.

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Following Intracochlear Administration of AK-OTOF, No Impact on Clinical, Otic, or Systemic Pathology was Observed

- Clinical pathology assessments in NHPs were performed by an independent contract research organization
- Histopathology evaluations in NHPs were performed by an independent, board-certified veterinary pathologist; brain histopathology evaluations in mice were performed by independent certified pathology services

	NHPs *bilateral administration of AK-OTOF at Dose 2.5X, 5X, 9X, or 15X*	*Otof $^{-/-}$ Mice* *unilateral administration of AK-OTOF at Dose 1X, 2.25X, or 5X*
Otic, Systemic, Clinical Pathology	No adverse findings related to intracochlear administration of AK-OTOF through longest duration evaluated (6 months post-administration) for:	
	• Auditory / cochlear function (ABRs and DPOAEs) • Macro- or microscopic otic histopathology • Brain histopathology • Organ weights • Hematology, coagulation, serum chemistry • Urinalysis	• Auditory / cochlear function (ABRs and DPOAEs)* • Cochlear histology* • Brain histopathology • Organ weights* • Gross pathology [Clinical pathology was not performed]

* Evaluations were conducted by Akouos personnel.

Abbreviations: ABRs = auditory brainstem responses; DPOAEs = distortion product otoacoustic emissions; NHPs = non=human primates; *Otof* $^{-/-}$ = otoferlin knock-out.

Summary

- Individuals with *OTOF*-mediated hearing loss typically have absent ABRs and are likely to experience a decline in cochlear integrity typically within the first decade of life, indicated by initially present, then absent, OAEs
- The *Otof* $^{-/-}$ mouse model used in efficacy studies 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 the human population
- Intracochlear administration of AK-OTOF in *Otof* $^{-/-}$ mice prior to decline in OAEs restores auditory function as early as Day 15 post-administration; restoration is durable through at least 6 months
    - These data suggest that humans with present OAEs may receive the most potential benefit from AK-OTOF
- AK-OTOF has a robust safety profile: intracochlear administration was locally and systemically well tolerated in NHPs and mice
- Together, these IND-enabling nonclinical studies support the planned clinical development of AK-OTOF for the treatment of *OTOF*-mediated hearing loss

Akouos Received FDA Clearance of its Investigational New Drug (IND) Application for AK-OTOF

- In September 2022, the IND for AK-OTOF received FDA clearance to evaluate AK-OTOF for the treatment of otoferlin gene (*OTOF*)-mediated hearing loss
- Akouos plans to initiate a pediatric Phase 1/2 clinical trial (AK-OTOF-101), including children as young as two years of age in the dose-escalation phase (Part A)
- Important eligibility criteria include:
  - Biallelic otoferlin gene mutations
  - Absent ABRs
  - Present OAEs
- Akouos is currently conducting a natural history study (AK-OTOF-NHS-002) in individuals with *OTOF*-mediated hearing loss

For more information, please refer to www.clinicaltrials.gov using the search term “Akouos”

Abbreviations: ABRs = auditory brainstem responses; FDA = U.S. Food and Drug Administration; OAEs = otoacoustic emissions; *OTOF* = human otoferlin gene.

Thank you!