Nonclinical In Vivo Expression, Durability of Effect, Biodistribution/Shedding, and Safety Evaluations Support Planned Clinical Development of AK-OTOF (AAVAnc80-hOTOF Vector) for OTOF-*mediated Hearing Loss*

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Akouos, Inc., Boston, MA

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Disclosures

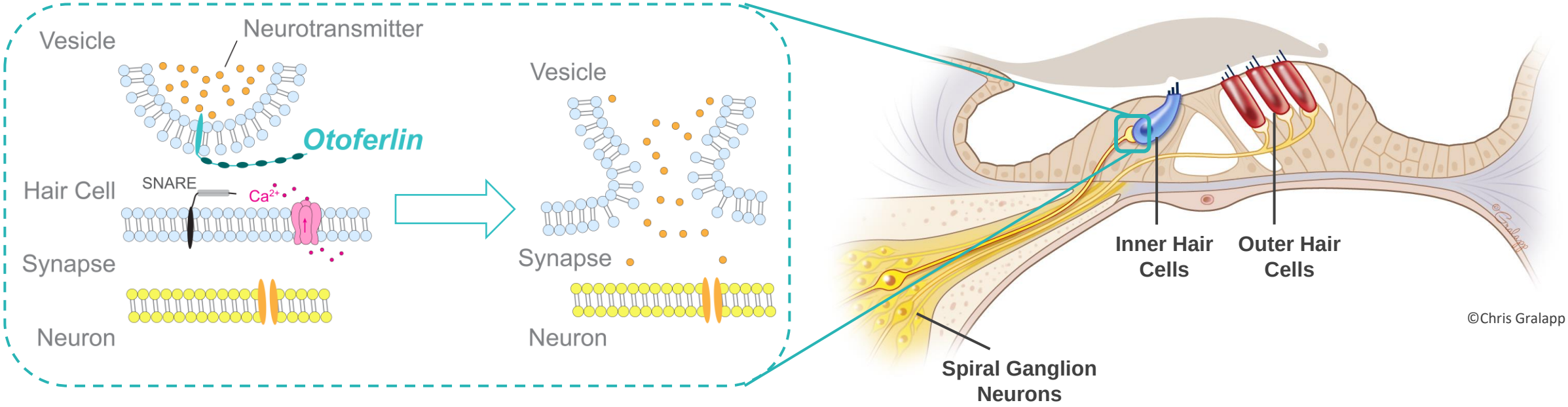
Ann Hickox is an employee of Akouos, Inc., and has received, and is receiving, compensation and equity from Akouos, Inc.

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; and statements relating to the initiation, plans, and timing of our future clinical trials and our research and development programs. 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 our Quarterly Report on Form 10-Q for the quarter ended March 31, 2022, 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 otoferlin gene *(OTOF)* 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

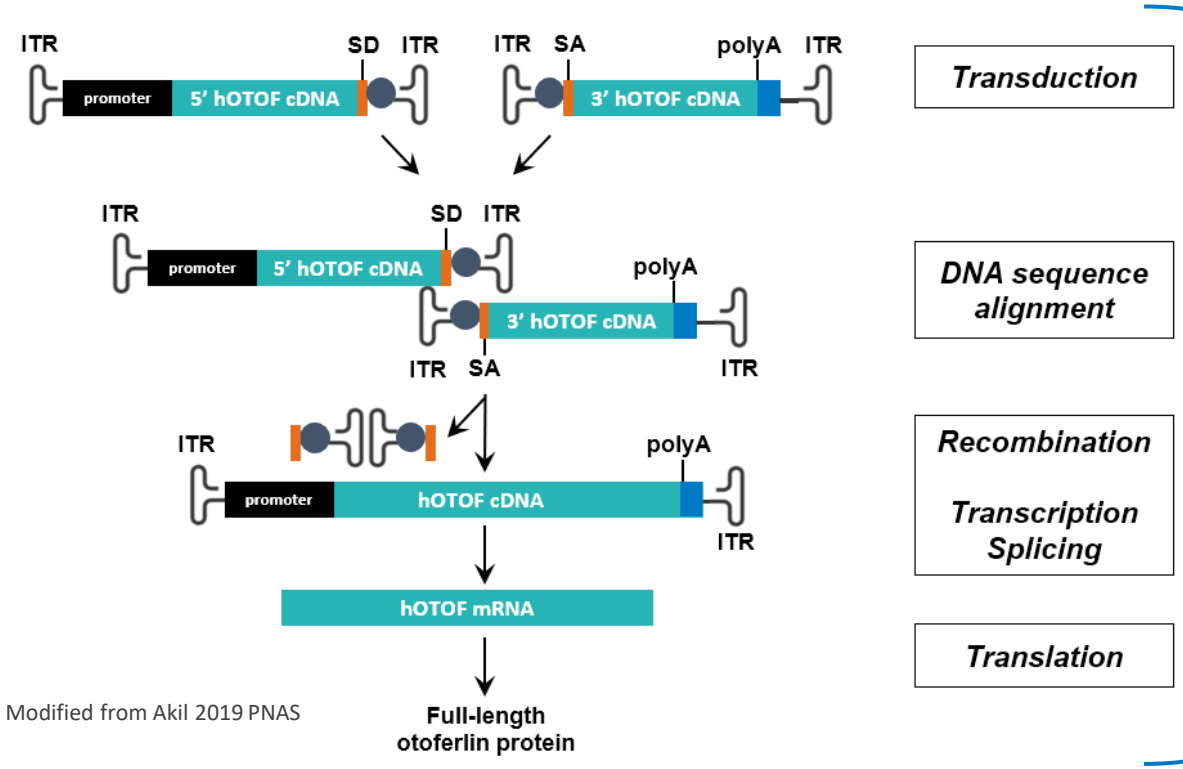


- *OTOF*-mediated hearing loss is a form of sensorineural hearing loss (SNHL) that typically presents as a Severe to Profound, bilateral, congenital form of SNHL caused by biallelic mutations in *OTOF*

(Yasunaga 1999 Nat Genet; Pangršič 2012 Trends Neurosci)

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

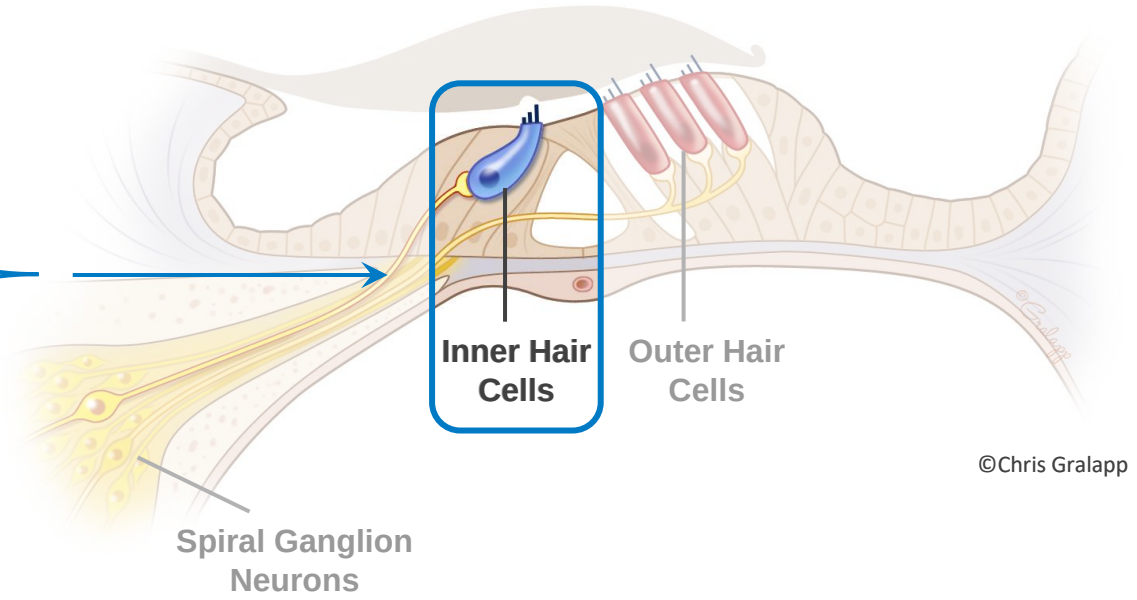
DNA sequence alignment

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

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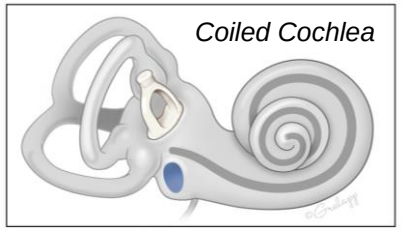
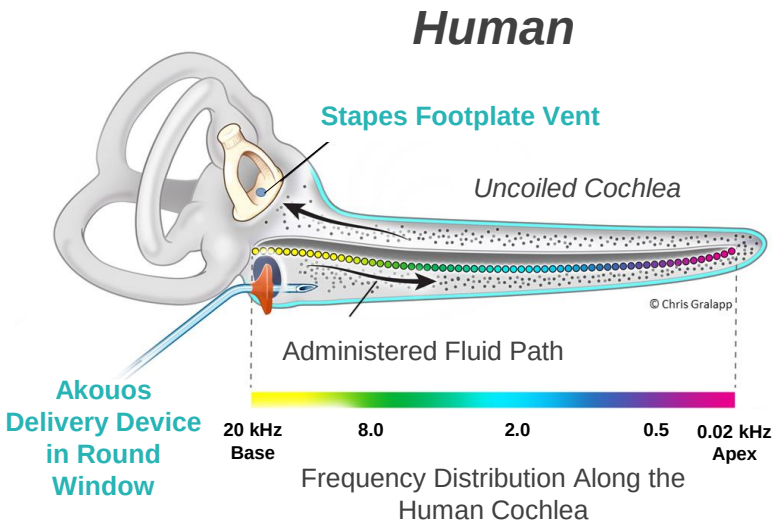
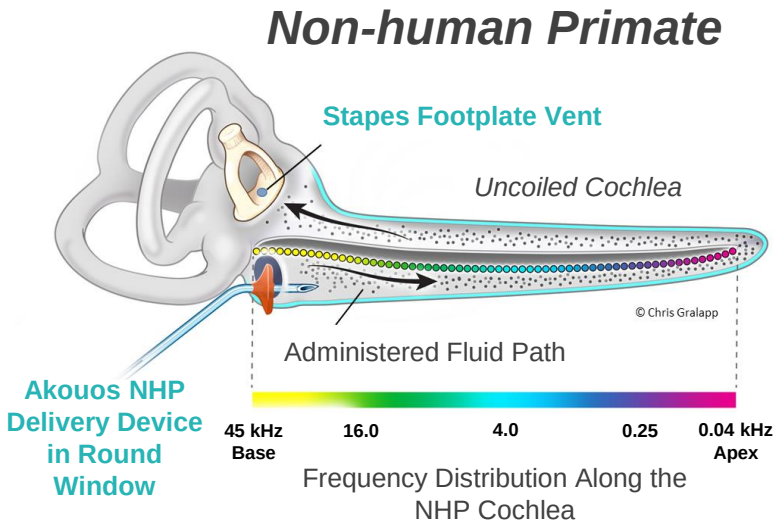
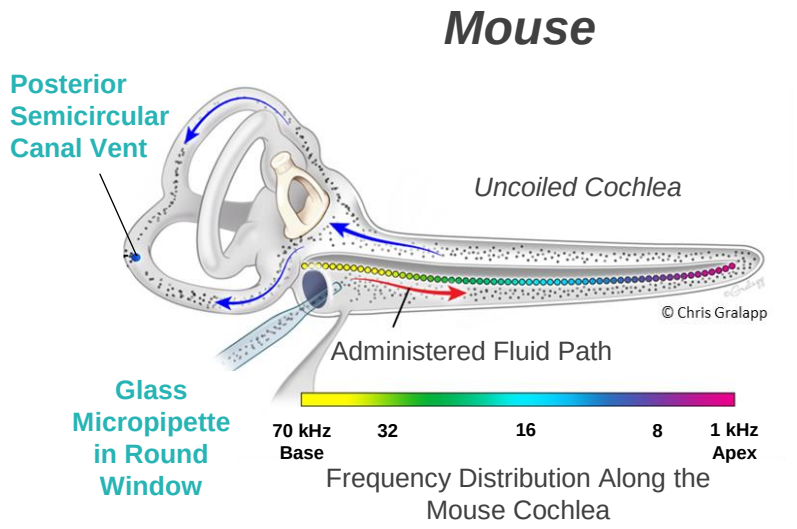
IHCs: inner hair cells; SGNs: spiral ganglion neurons

Intracochlear Delivery of AK-OTOF is Similar Across Mammalian Species







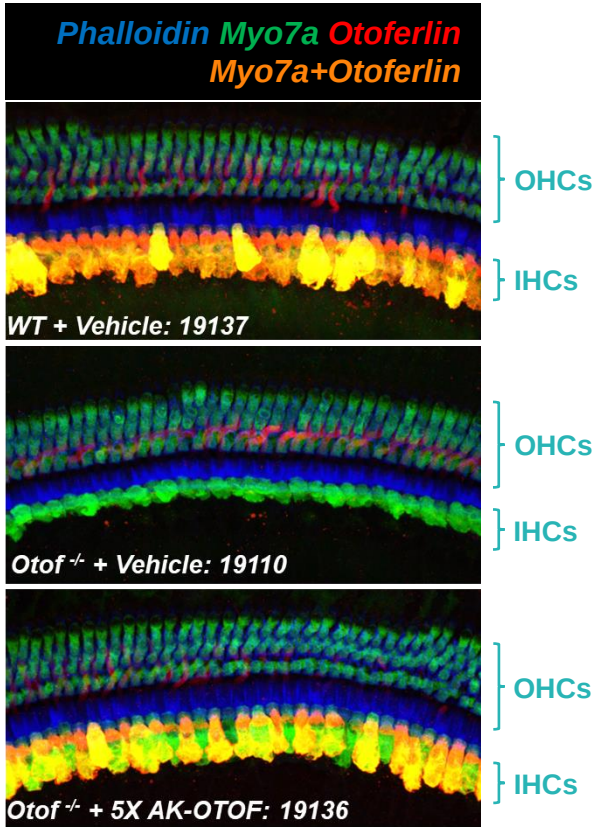


- The drug delivery process to the cochlea is similar across human, non-human primates (NHPs), and mice, i.e., delivery to the intracochlear space via the round window membrane accompanied by venting / fenestration
- Creation of a fenestration, or vent, allows for distribution of therapeutic fluids along the length of the cochlea and also serves to prevent a potential deleterious rise in pressure during administration
- The surgical approach to access the cochlea is modified to accommodate differences in species anatomy surrounding the cochlea
- The dose of AK-OTOF is scaled across species based on relative inner ear volume (keeping vector concentration consistent); throughout this presentation, dose levels are presented as relative values based on concentration to normalize across species

Throughout this presentation, NHPs refers to cynomolgus macaques.

Expression of Full-length Human Otoferlin Protein was Observed Only in the Target Inner Hair Cells

Micrographs from Mid-cochlear Region in Mice 1 Month Post-administration of vehicle or AK-OTOF

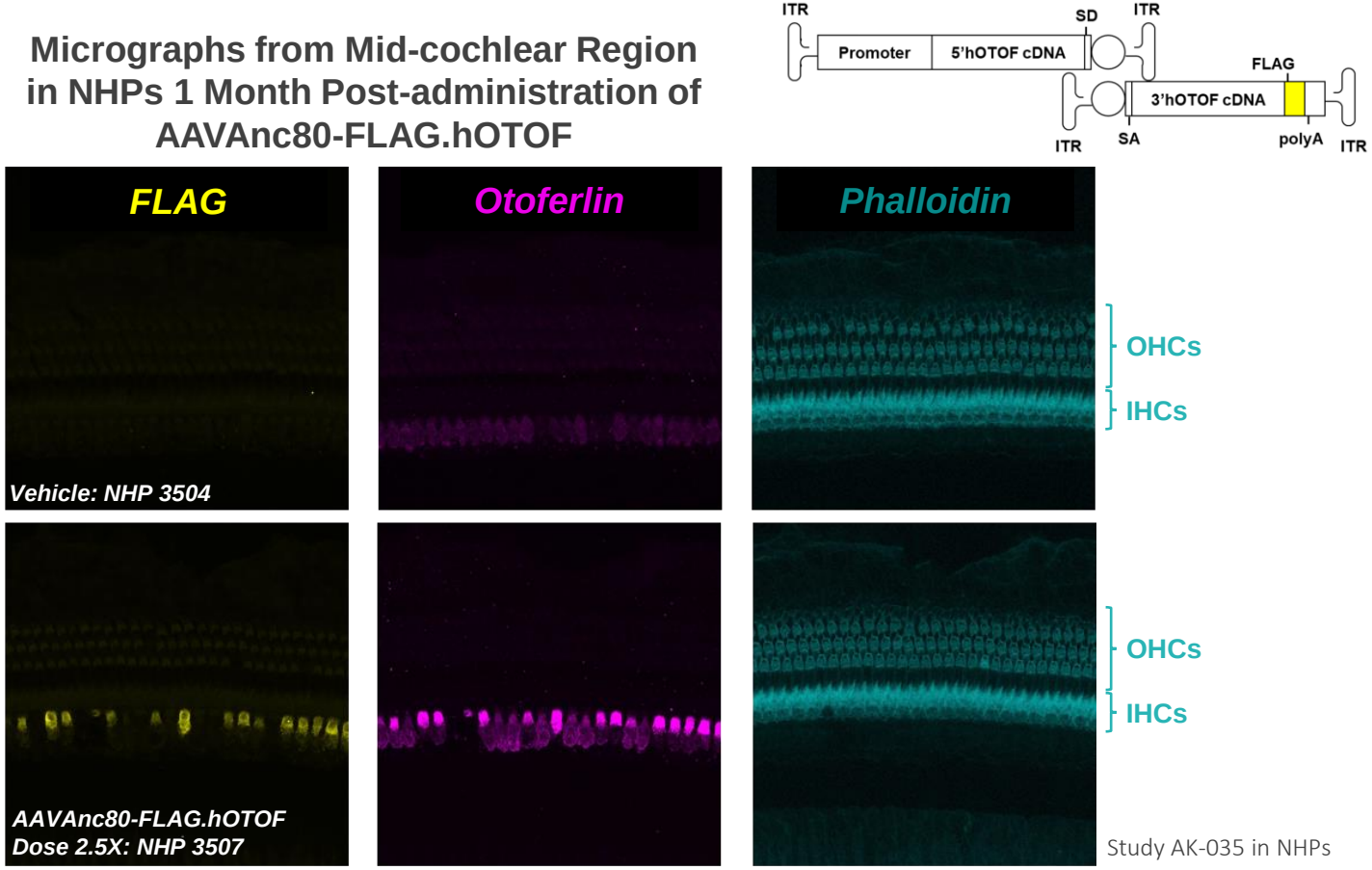


Wild-type otoferlin expression in IHCs

Restored otoferlin expression in IHCs of *Otof* $^{-/-}$ mouse

Study AK-019 in mice

Micrographs from Mid-cochlear Region in NHPs 1 Month Post-administration of AAVAnc80-FLAG.hOTOF



Study AK-035 in NHPs

No AAVAnc80-mediated expression of otoferlin-FLAG (assessed by co-staining of FLAG and otoferlin) was identified in other cochlear neural regions or supporting cell regions evaluated.

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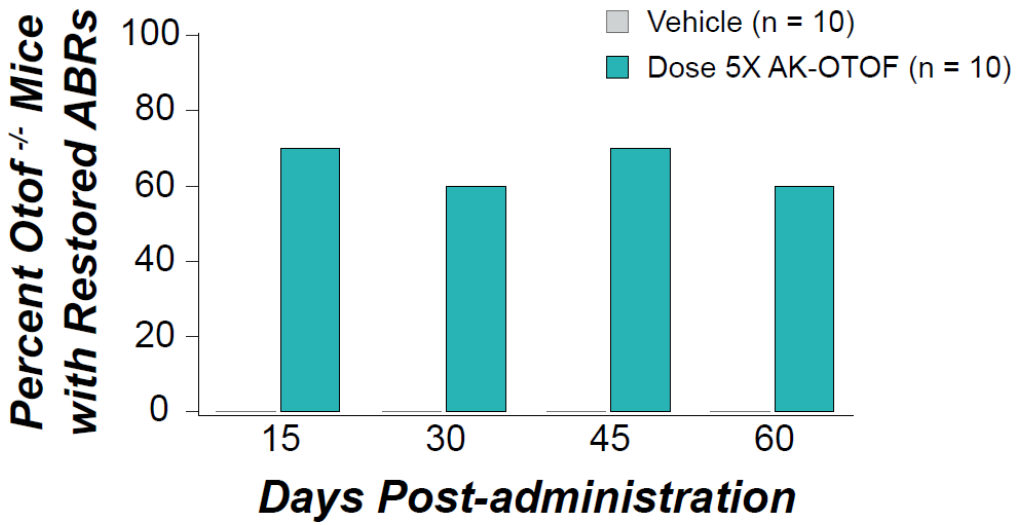
Non-specific labelling of pillar cells (overlapping herein with OHC region) is seen in otoferlin channel (red), irrespective of genotype or article administered

Cochlear micrographs represent maximum projections through confocal image stacks; IHCs = inner hair cells; NHP = non-human primate; OHC = outer hair cells; *Otof* $^{-/-}$ = otoferlin knockout; WT = wild-type.

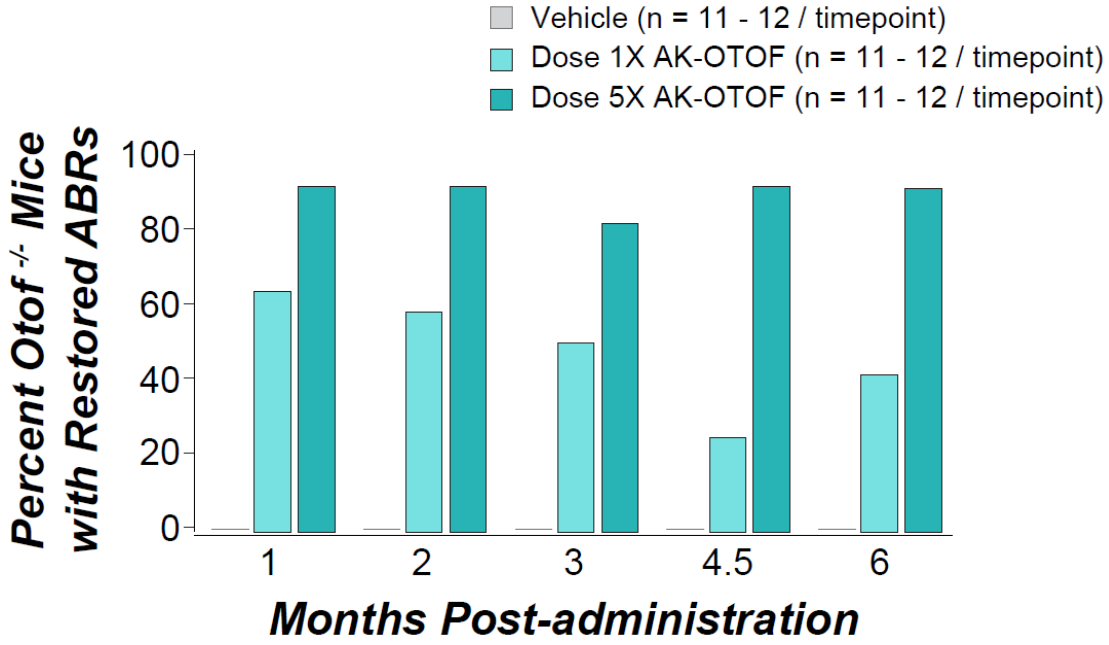
Expression of Human Otoferlin Protein in Inner Hair Cells Restored Auditory Function in Otoferlin Knockout (*Otof* $^{-/-}$) Mice

- Auditory function was evaluated in mice using the auditory brainstem response (ABR), an electrophysiologic audiometry assessment used clinically and in nonclinical models
- Unlike *Otof* $^{-/-}$ mice administered vehicle, which have no measurable ABRs, approximately 70% of *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 5X showed restored ABRs by Day 15



Study AK-026 in *Otof* $^{-/-}$ mice

- At least 80% of *Otof* $^{-/-}$ mice administered AK-OTOF at Dose 5X had restored ABRs through at least 6 months (the longest survival duration evaluated)
- The extent of auditory function restoration was dependent on the dose administered

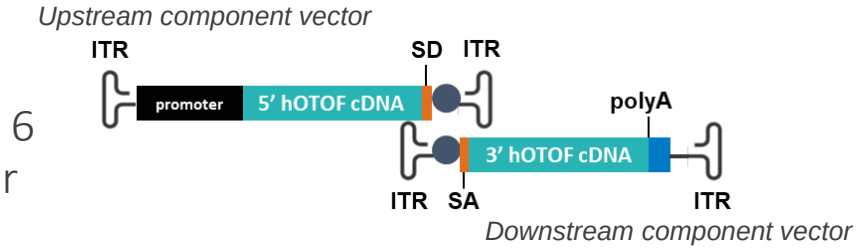


Study AK-019 in *Otof* $^{-/-}$ mice

Restored ABR = Click-evoked ABR threshold within the range of vehicle-injected wild-type mice.

Detection of AK-OTOF Vector Sequences Persisted in the Cochlea, but Occurred Less Frequently and Decreased Rapidly in Non-cochlear Tissues and Fluids

- Two validated qPCR assays were used to quantify biodistribution and vector shedding of each of the upstream and downstream component vectors through 6 months (the longest survival duration evaluated) following bilateral intracochlear administration of AK-OTOF in non-human primates (NHPs)



Biodistribution / Shedding in Fluid / Swabs (Dose 2.5X, 5X, or 9X of AK-OTOF)

- Serum, blood, urine, and saliva / nasal swab samples tested below the limit of detection by approximately Week 3 to 4 post-administration
- Component vector sequences were detected in approximately 70% of ear swab samples at Day 3; the latest time point with positive samples was Month 3 (detected in approximately 20% of ear swabs), with no positive samples at Months 4, 5, or 6

Study AK-042 in NHPs

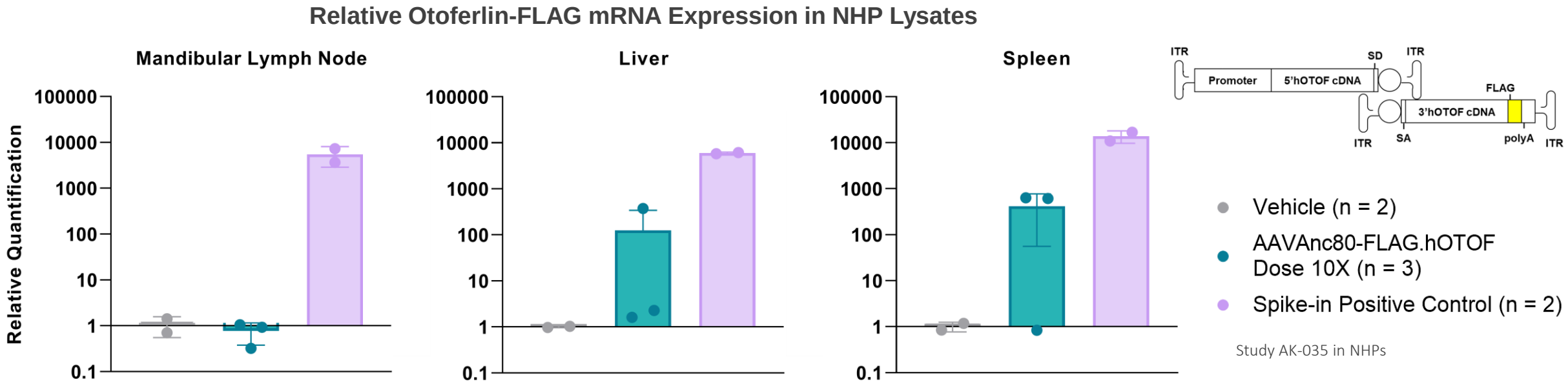
Biodistribution to Tissues (Dose 2.5X, 5X, 9X, or 15X of AK-OTOF)

- Component vector sequences were detected in the target tissue (cochlea) through Month 6
- The majority of evaluated tissue types tested below the limit of detection / quantitation for sequences of both component vectors
- Component vector sequences were detected through Month 6 in liver, spleen, and lymph nodes, decreasing in copy number by Month 6

Study AK-009 / Study AK-042 in NHPs

Minimal Otoferlin-FLAG mRNA Expression was Detected in Non-target Tissues Following Intracochlear Administration of AAVAnc80-FLAG.hOTOF

- Use of a tagged vector (AAVAnc80-FLAG.hOTOF) in non-human primates (NHPs) differentiated vector-mediated *vs.* endogenous otoferlin expression in the target cochlea and was also used to evaluate expression in non-target tissues
- Based on biodistribution results following intracochlear administration in NHPs, non-target tissue types that had detectable AK-OTOF vector sequences were evaluated for potential human otoferlin expression one month following bilateral intracochlear administration
- Only liver and spleen were positive for human otoferlin-FLAG mRNA expression by RT-qPCR, and only in a proportion of animals

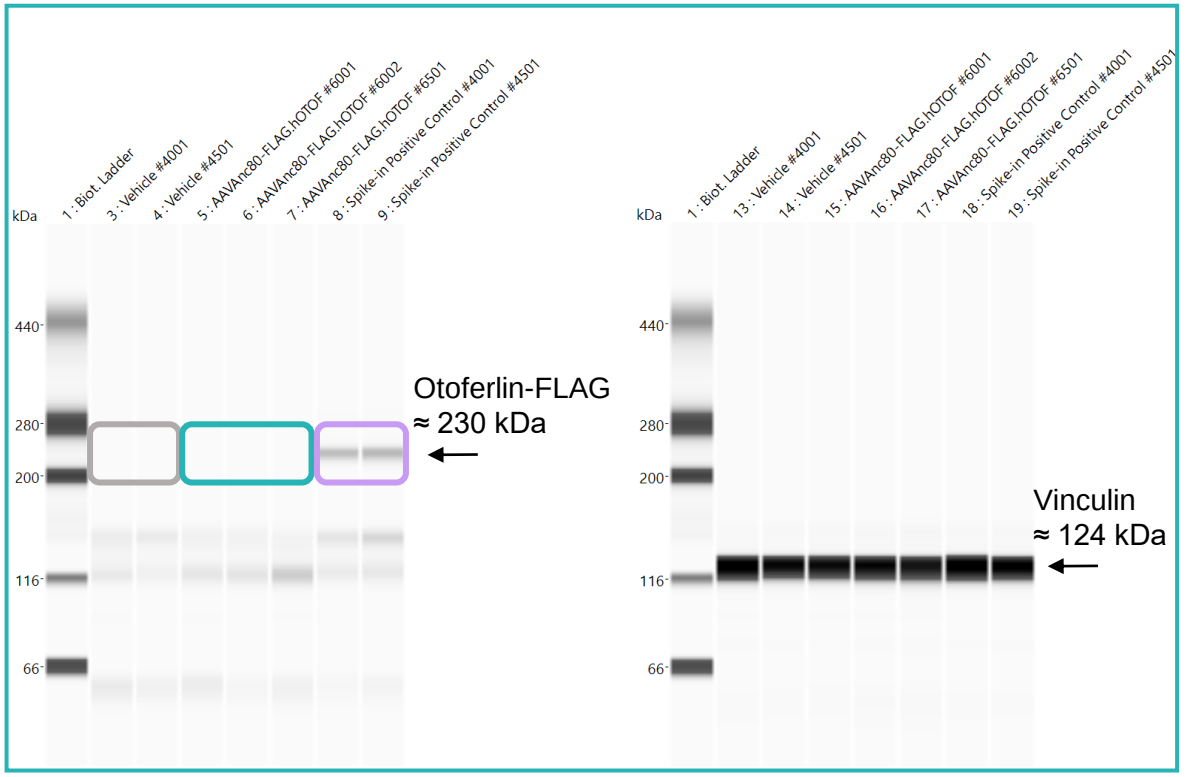


Spike-in positive control = Cell transduction control lysate added to NHP tissue lysate; Cell transduction control = Transduced HEK293FT cells lysate (not shown).

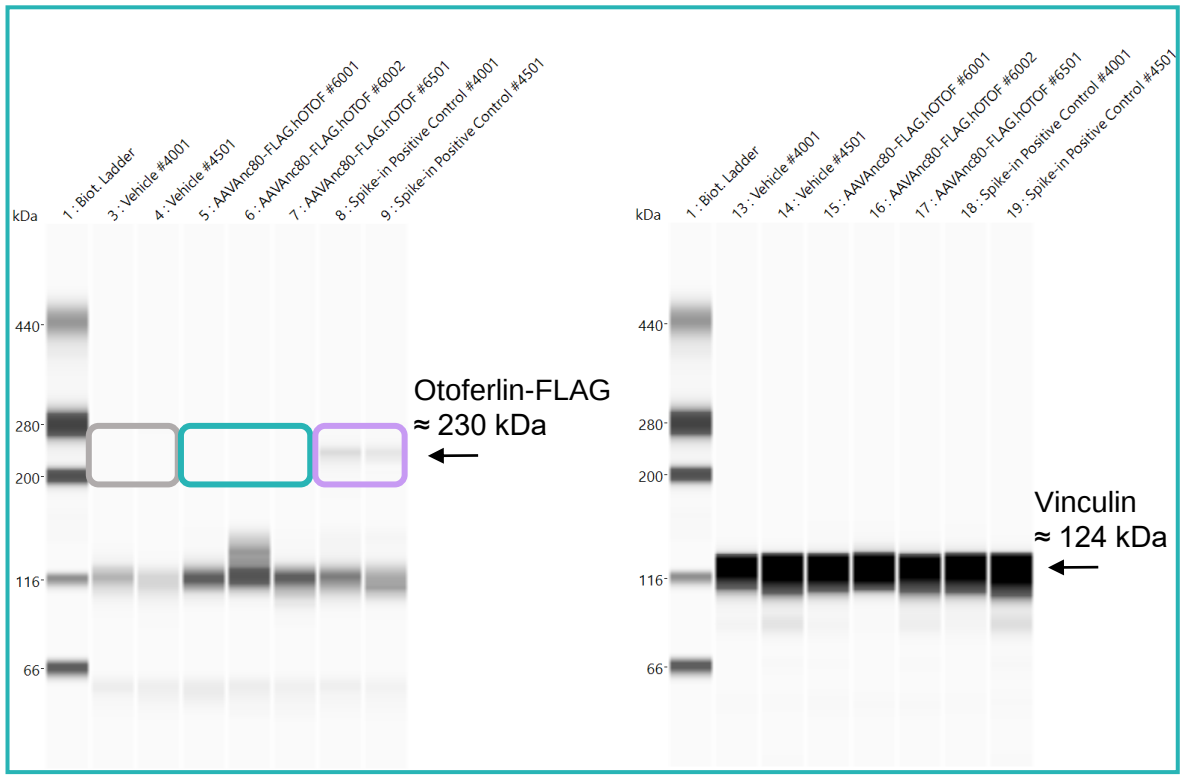
Similar relative quantification between cell transduction control and spike-in positive control indicated no tissue matrix effect. Mean ± SD shown.

Otoferlin-FLAG Protein Expression was Not Detected in Non-target Tissues Following Intracochlear Administration of AAVAnc80-FLAG.hOTOF

Wes™ Blots From NHP Liver Lysates



Wes™ Blots From NHP Spleen Lysates



- Vehicle (n = 2)
- AAVAnc80-FLAG.hOTOF Dose 10X (n = 3)
- Spike-in Positive Control (n = 2)

Low molecular weight bands that are visible for all groups, including vehicle, represent non-specific binding in NHP lysate matrices and not truncated otoferlin proteins; no detectable truncated otoferlin proteins were observed when HEK293FT cells were transduced with AK-OTOF (Andres-Mateos, ASGCT 2021).

Study AK-035 in NHPs

Spike-in positive control = Cell transduction control lysate added to NHP tissue lysate; Cell transduction control = Transduced HEK293FT cells lysate (not shown).

Similar relative quantification between cell transduction control and spike-in positive control indicated no tissue matrix effect.

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

- Clinical pathology assessments of safety / tolerability in non-human primates (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

Evaluations in NHPs (bilateral administration)
(Dose 2.5X, 5X, 9X, or 15X of AK-OTOF)

Clinical Pathology

- No changes in hematology, coagulation, serum chemistry, or urinalysis related to intracochlear administration of AK-OTOF through 6 months post-administration (longest duration evaluated)

Otic and Systemic Pathology

- No findings in macroscopic or microscopic otic histopathology, brain histopathology, or organ weights related to intracochlear administration of AK-OTOF through 6 months post-administration

Study AK-009 / Study AK-042 in NHPs

Evaluations in Otof $^{-/-}$ *Mice*
(unilateral administration)
(Dose 1X, 2.25X, and 5X of AK-OTOF)

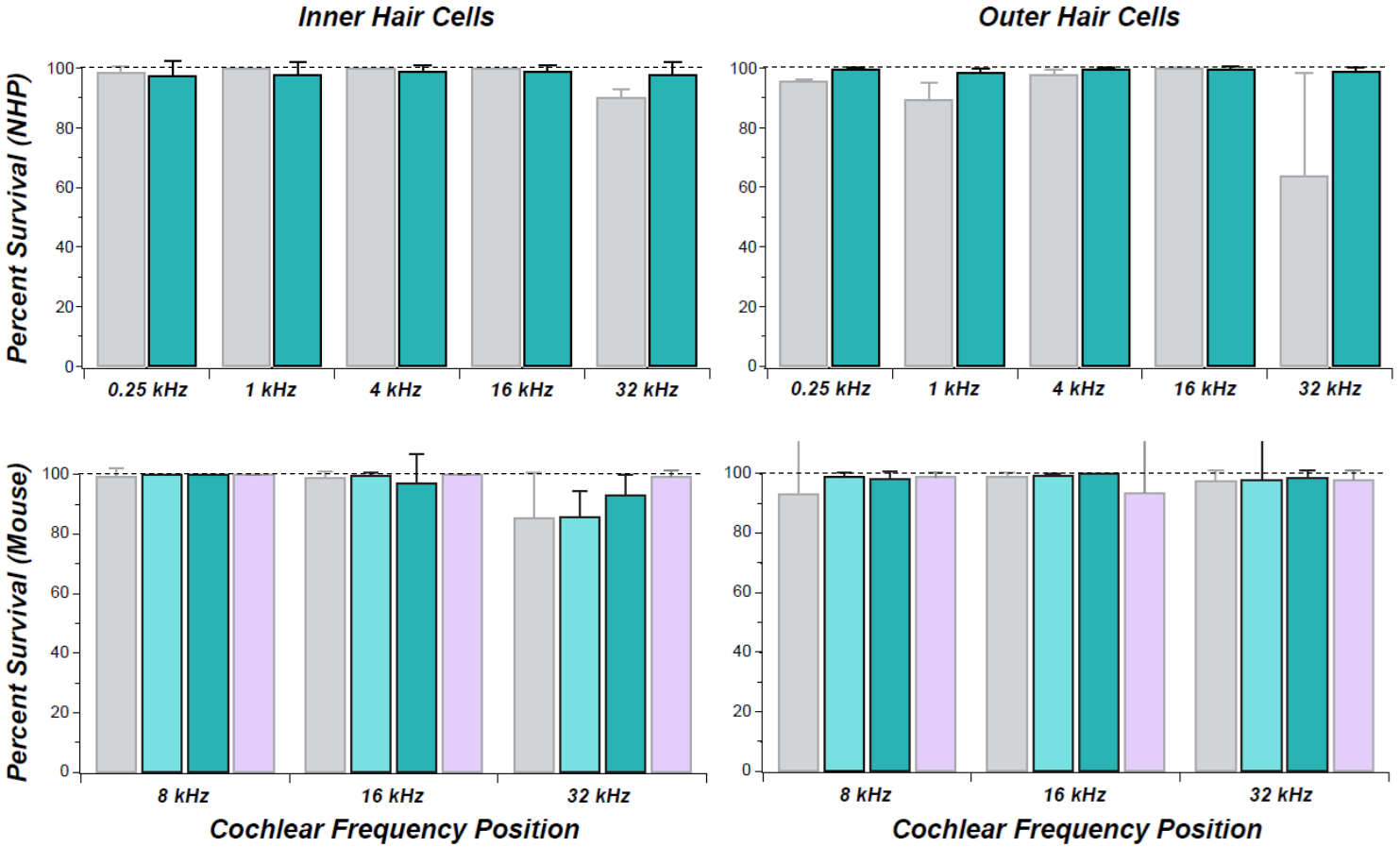
Otic and Systemic Pathology

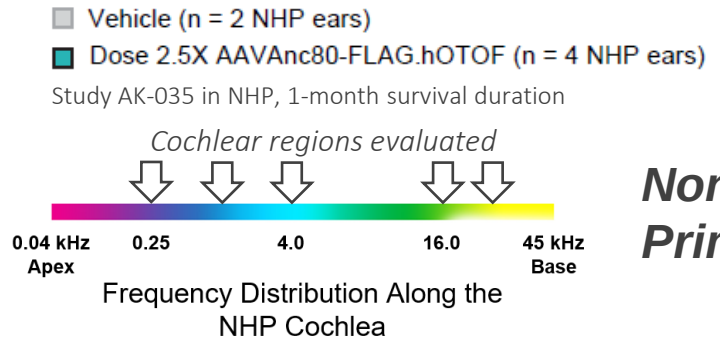
- No adverse systemic or otic effects were observed
- No findings in brain histopathology related to administration of AK-OTOF through 6 months post-administration (longest duration evaluated)

Study AK-019 in *Otof* $^{-/-}$ mice

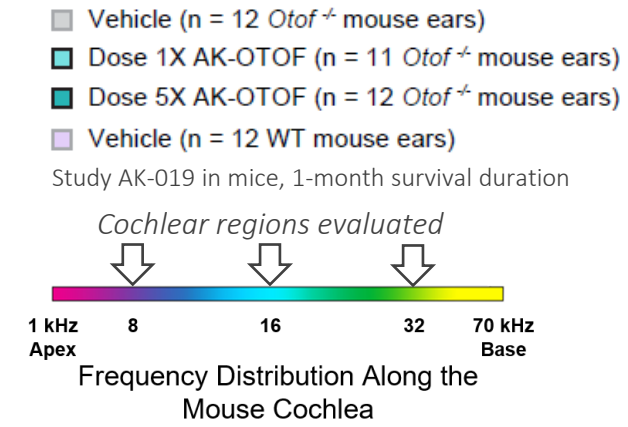
Following Intracochlear Administration of AK-OTOF or AAVAnc80-FLAG.hOTOF, No Impact on Cochlear Hair Cell Survival was Observed

- Cochlear hair cell survival was quantified to assess local tolerability of intracochlear administration of either AAVAnc80-FLAG.hOTOF to non-human primates (NHPs) or AK-OTOF to *Otof* $^{-/-}$ mice; hair cell survival was robust throughout the cochlea and similar to vehicle-injected NHPs or to vehicle-injected *Otof* $^{-/-}$ mice and wild-type mice, respectively





Non-human Primate

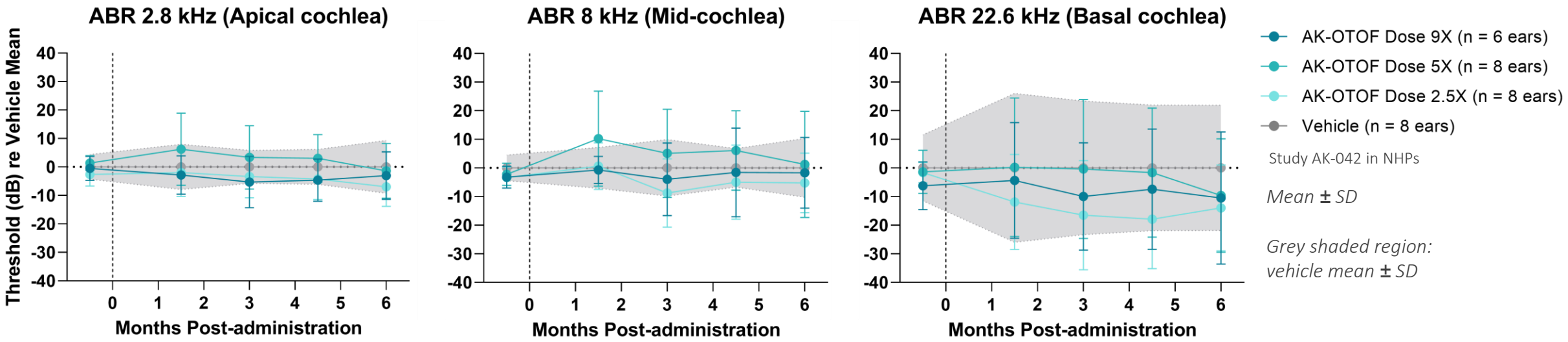


Mouse

Otof $^{-/-}$ = otoferlin knockout; WT = wild-type. Mean ± SD shown.

Following Intracochlear Administration of AK-OTOF in Non-human Primates, No Impact on Auditory / Cochlear Function was Observed

- Auditory function (auditory brainstem response [ABR]) was evaluated pre- and post-intracochlear administration of AK-OTOF in non-human primates (NHPs) to assess local tolerability
- No impact of AK-OTOF, across a range of dose levels, was observed on ABR thresholds, which were comparable to thresholds of vehicle-injected ears through the 6-month study duration



- Auditory function results were comparable to cochlear function results (assessed via distortion product otoacoustic emission [DPOAE] testing, not shown)

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 otoferlin gene (*OTOF*) to inner hair cells (IHCs)
- Intracochlear administration of AK-OTOF in otoferlin knockout (*Otof* $^{-/-}$) mice, or its tagged version (AAVAnc80-FLAG.hOTOF) in non-human primates, leads to full-length human otoferlin protein expression only in the target IHCs
- Human otoferlin expression in IHCs of *Otof* $^{-/-}$ mice restores auditory function as early as Day 15 post-administration and is durable through at least 6 months
- Limited systemic exposure of AK-OTOF following intracochlear administration was observed and tended to clear rapidly, and no otoferlin protein expression was detected for the non-target tissue types that showed minimal otoferlin mRNA expression
- AK-OTOF was systemically and locally well tolerated in both mice and NHPs, and no adverse effects were observed in clinical pathology, otic pathology, systemic histopathology, or auditory or cochlear function
- Together, these IND-enabling nonclinical studies support the planned clinical development of AK-OTOF for the treatment of *OTOF*-mediated hearing loss

Thank you!