Demonstration of Secreted Protein Expression Levels Following Intracochlear Delivery of AK-antiVEGF (AAVAnc80-antiVEGF Vector) Across Multiple Doses in Non-human Primates

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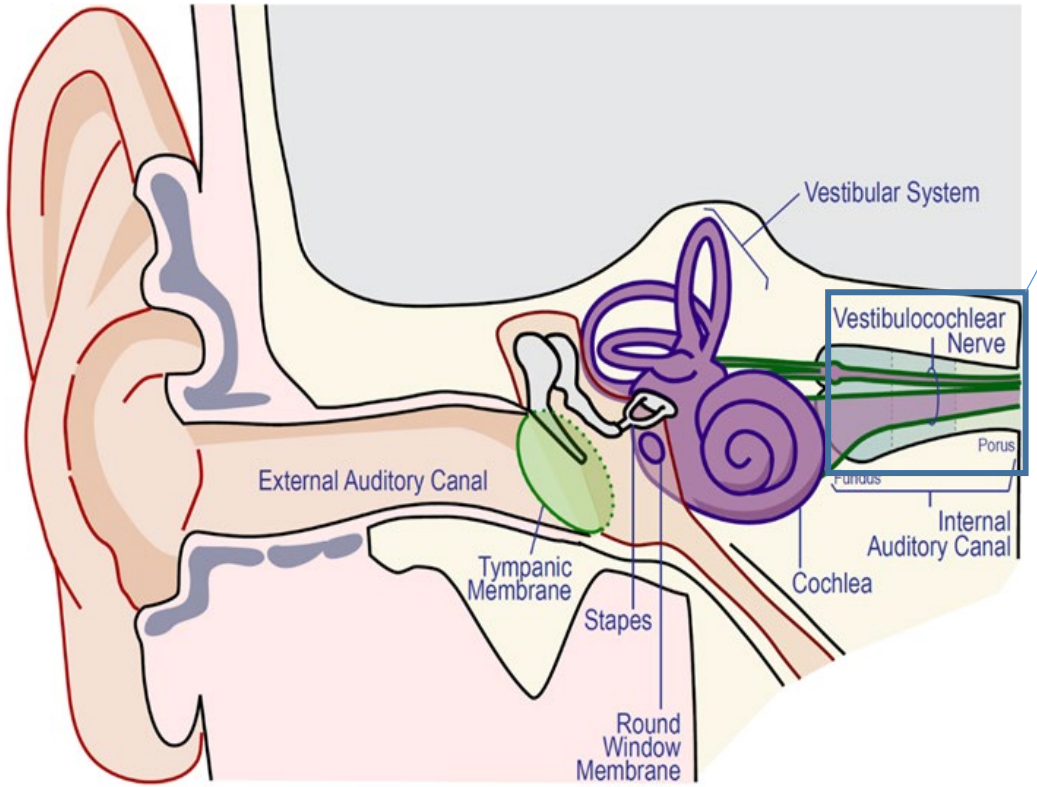
Forward Looking Statements

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-antiVEGF to potentially treat vestibular schwannoma; statements relating to the initiation, plans, and timing of our future clinical trials and our research and development programs; and the timing of our planned IND submission for AK-antiVEGF. 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 Quarterly Report on Form 10-Q for the quarter ended November 12, 2021, 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.

Vestibular Schwannoma

- Vestibular schwannomas (VSs) are tumors arising from Schwann cells that ensheathe the vestibulocochlear nerve
- VS is estimated to affect approximately 200,000 individuals in the US and Europe
- VS symptoms include hearing loss, tinnitus, headaches, and impaired balance, and could progress to additional co-morbidities
- Current standard of care is observation, surgical resection, and/or radiation therapy
- Vascular endothelial growth factor (VEGF) is upregulated in VS, including non-neurofibromatosis type 2 (non-NF2, sporadic) tumors[1]



VS most commonly occurs along the vestibulocochlear nerve as it courses through the internal auditory canal (IAC) to the brainstem. The majority of small, intracanalicular tumors (less than 5 mm width) arise within the lateral third of the IAC, nearest to the cochlea[2]

Image modified from:
https://commons.wikimedia.org/wiki/File:Anatomy_of_the_Human_Ear.svg

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[1]Koutsimpelas D, et al. *ORL J Otorhinolaryngol Relat Spec.* 2012;74(1):33-37, [2]Koen N, et al. *Otolaryngol Head Neck Surg* 2020; 162(2):211-214

VEGF Inhibition Shows Promise in *NF2*-related VS, but Systemic Administration Can Result in Toxicity

Clinical Trial Data Demonstrate Ability of Systemic VEGF Inhibitor to Improve Hearing and Reduce Tumor Volume in Some Patients With VS

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Hearing Improvement after Bevacizumab in Patients with Neurofibromatosis Type 2

Scott R. Plotkin, M.D., Ph.D., Anat O. Stemmer-Rachamimov, M.D., Fred G. Barker II, M.D., Chris Halpin, Ph.D., Timothy P. Padera, Ph.D., Alex Tyrrell, Ph.D., A... and

Neuro-Oncology 12(1):14–18, 2010.
doi:10.1093/neuonc/nop010
Advance Access publication October 20, 2009

NEURO-ONCOLOGY

Bevacizumab induces regression of vestibular schwannomas in patients with neurofibromatosis type 2†

Victor-Felix Mautner, Rosa Nguyen, Hannes Kutta, Carsten Fuensterer, Carsten Bokemeyer, Christian Hagel, Reinhard E. Friedrich, and Jens Panse

Department of Maxillofacial Surgery (V.M., R.N., R.E.F.); Department of Otolaryngology (H.K.); Institute of Neuropathology (C.H.); Department of Oncology/Hematology, Cancer Center (J.P., C.B.); University Medical Center Hamburg-Eppendorf, Hamburg, Germany; MRI Institute, Hamburg Othmarschen, Germany (C.F.)

J Neurooncol
DOI 10.1007/s11060-015-1828-8

CLINICAL STUDY

Bevacizumab decreases vestibular schwannomas growth rate in children and teenagers with neurofibromatosis type 2

Audrey Hochart[1] · Vianney Gaillard[2] · Marc Baroncini[3] · Nicolas André[4,5] · Jean-Pierre Vannier[6] · Matthieu Vinchon[3] · Frederique Dubrulle[2] · Jean-Paul Lejeune[3] · Christophe Vincent[7] · Véronique Nève[8] · Hélène Sudour Bonnange[1] · Nicolas Xavier Bonne[7] · Pierre Leblond[1]

Meta-analysis: Clinical Trials of Efficacy and Safety of Bevacizumab in Patients With *NF2*-related VS

Journal of Neuro-Oncology (2019) 144:239–248
https://doi.org/10.1007/s11060-019-03234-8

TOPIC REVIEW

Efficacy and safety of bevacizumab for vestibular schwannoma in neurofibromatosis type 2: a systematic review and meta-analysis of treatment outcomes

Victor M. Lu[1] · Krishnan Ravindran[1] · Christopher S. Graffeo[1] · Avital Perry[1] · Jamie J. Van Gompel[1] · David J. Daniels[1] · Michael J. Link[1]

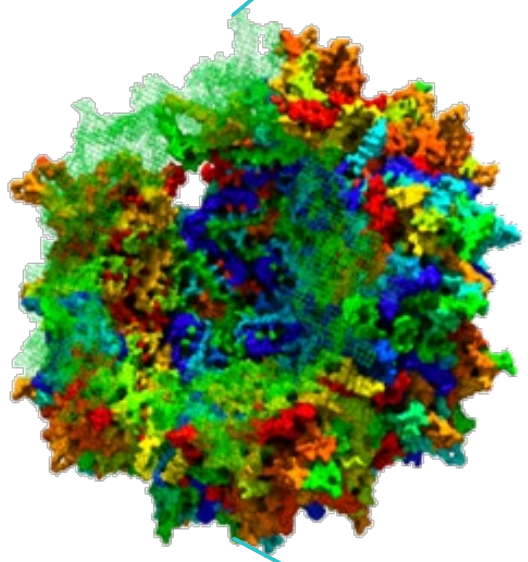
Outcome	Pooled incidence (95% CI)
Radiographic response	
Partial regression	41% (31–51%)
Stable	47% (39–55%)
Progression	7% (1–15%)
Hearing outcome	
Improvement	20% (9–33%)
Stable	69% (51–85%)
Worsening	6% (1–15%)

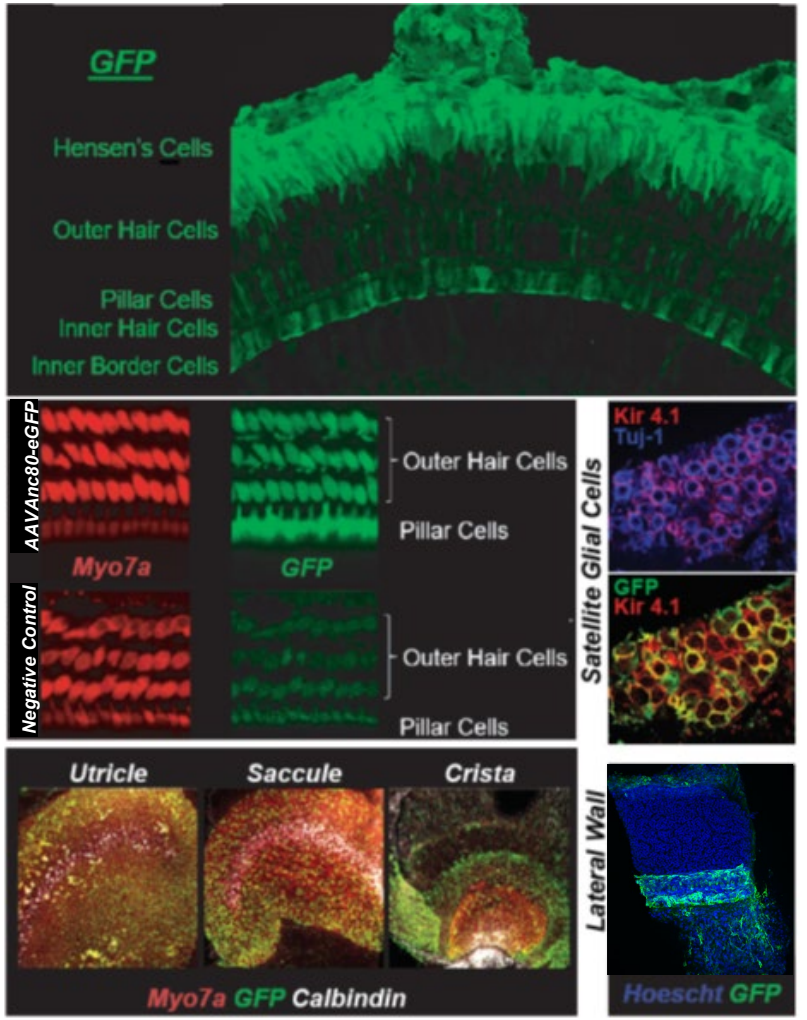
Outcome	Pooled incidence (95% CI)
Complications	
Serious toxicity	15% (10–26%)
Hypertension	33% (20–45%)
Proteinuria	43% (23–64%)
Amenorrhea	70% (51–87%)
Other	
Surgical interven-tion	11% (2–20%)

Abbreviations: NF2 = neurofibromatosis type 2; VEGF = vascular endothelial growth factor; VS = vestibular schwannoma(s).

AAVAnc80 Efficiently Transduces Multiple Cell Types in the Inner Ear

- ✓ Conducted nonclinical studies across three different species of non-human primates (NHP) using green fluorescent protein (GFP) as a reporter gene delivered by AAVAnc80
- ✓ AAVAnc80 can efficiently transduce multiple target cell populations throughout the cochlea in the primate inner ear
- ✓ In the planned approach, after administration of a vector utilizing a ubiquitous promoter, the cochlear and vestibular cells are expected to produce and secrete anti-VEGF protein into the perilymph

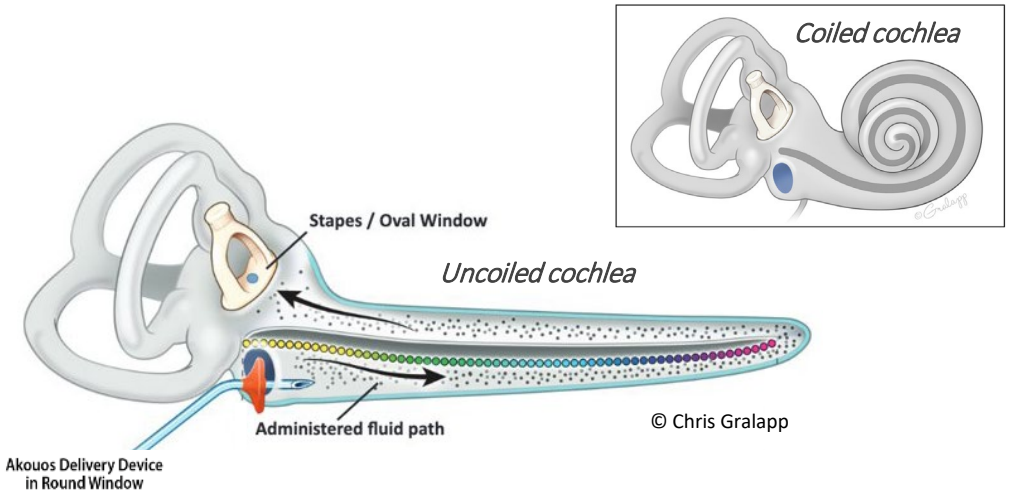


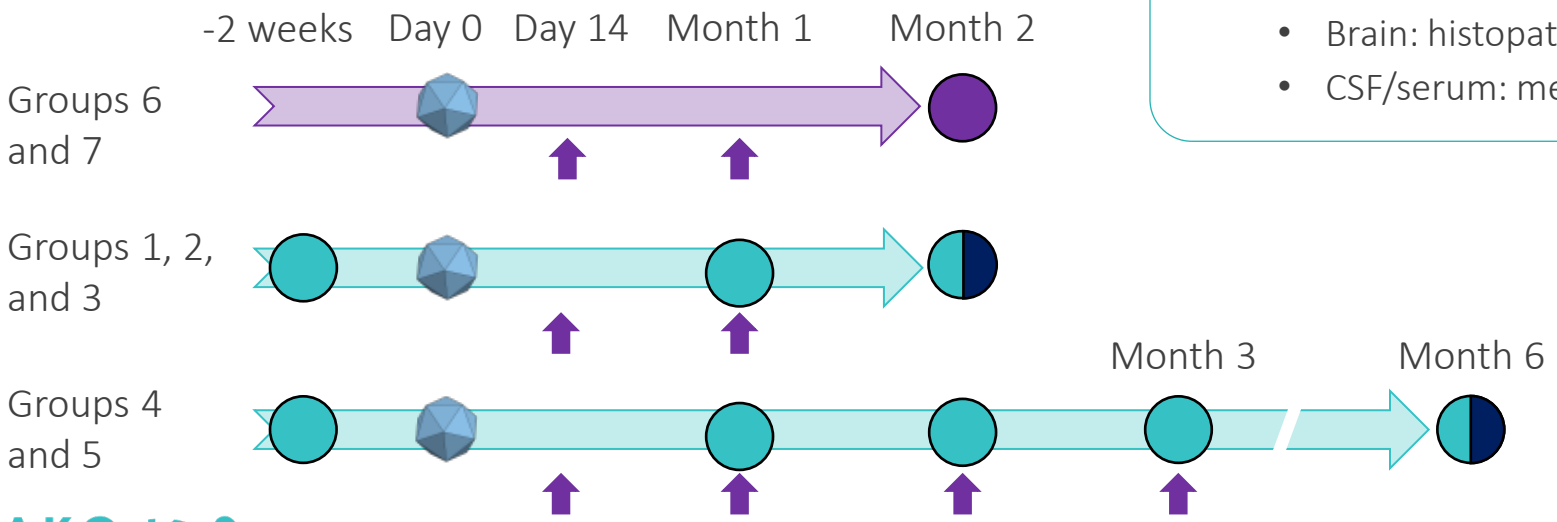


All micrographs are from Study AK-007, with the exception of Satellite Glial Cells from Study AK-011

Abbreviations: VEGF = vascular endothelial growth factor.

Preliminary Evaluation of Tolerability and Exposure of AK-antiVEGF in Non-human Primates (AK-033)





- Bilateral auditory function tests (ABR): 1.1, 2.8, 8.0, and 22.6 kHz
- Bilateral intracochlear administration of AK-antiVEGF (1X dose or 5X dose) or vehicle control
- In vivo serum collection for MSD assay measurement of anti-VEGF levels
- Terminal fluid and unfixed (flash frozen) tissue collection for MSD assay measurement of anti-VEGF levels
- Bilateral auditory function tests (ABR); and Terminal fluid and fixed-tissue analyses:
  - Inner ear: otic histopathology (ear 1) or cytocochleogram analyses (contralateral ear)
  - Brain: histopathology, with focus on auditory regions
  - CSF/serum: measurement of anti-VEGF levels

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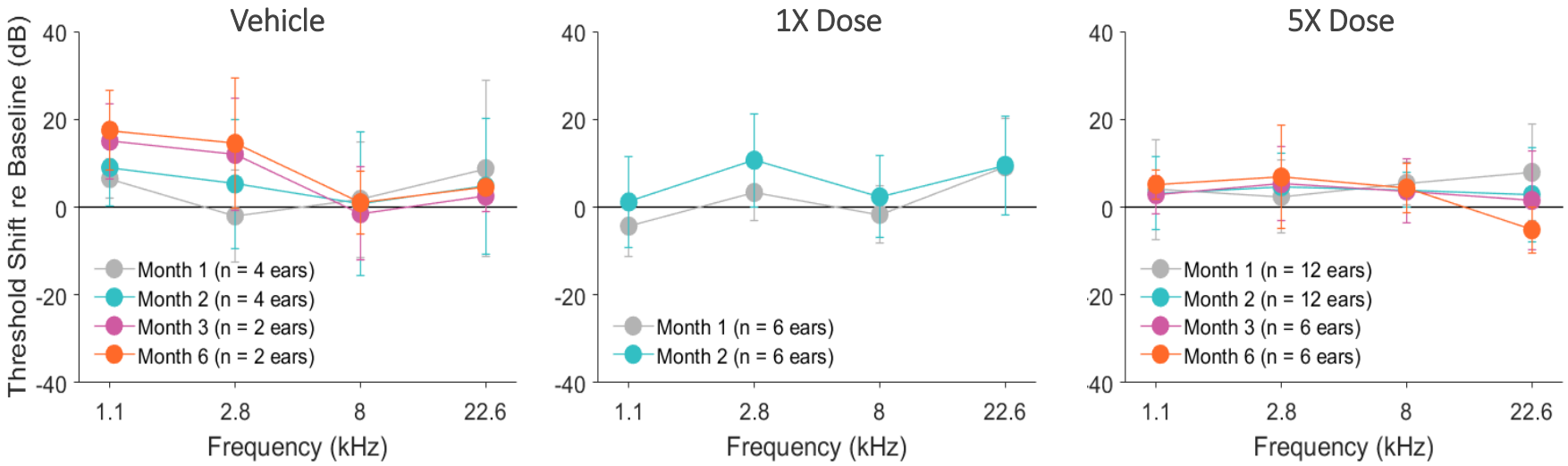
Abbreviations: ABR = auditory brainstem response; CSF = cerebrospinal fluid; kHz = kilohertz; MSD = meso-scale discovery; VEGF = vascular endothelial growth factor.

Physiologic and Histologic Evaluations Demonstrate AK-antiVEGF was Well Tolerated in NHPs (AK-033)



- Following administration of AK-antiVEGF at two doses, mean auditory brainstem response (ABR) thresholds at 1, 2, 3, and 6-month timepoints were similar to those at baseline
- Minimal ABR threshold shifts were likely attributable to the surgical approach used in NHPs
- Group means (±SD) at each timepoint reflect bilateral measurements in each NHP on study
- Cytocochleogram analyses revealed no signs of ototoxicity related to the test article
- No histopathology findings attributable to the test article

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Abbreviations: kHz = kilohertz; NHP = non-human primate(s); SD, standard deviation.

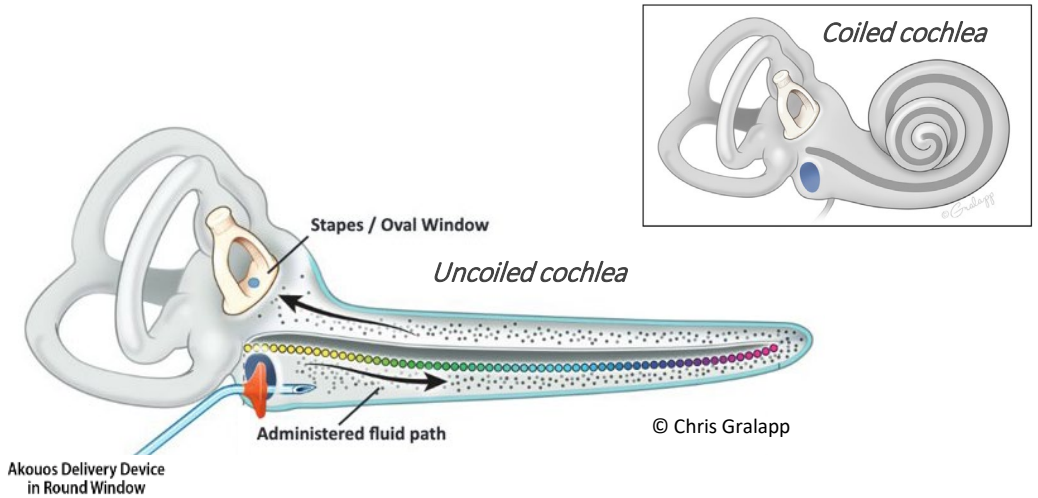
Anti-VEGF Protein Expression Evaluations in NHPs Support AK-antiVEGF Tolerability (AK-033)

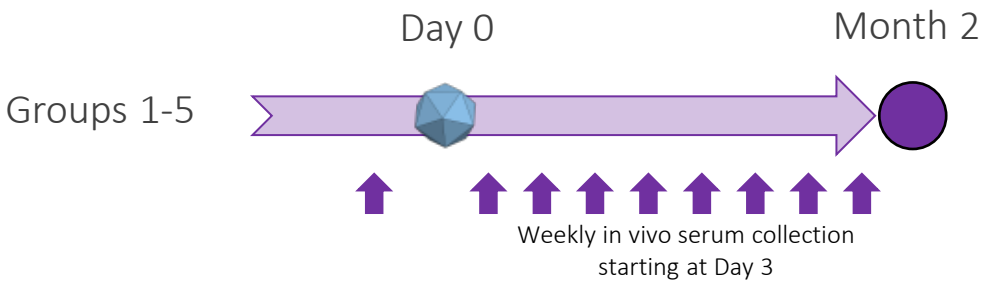
- Perilymph, serum, CSF, and tissue samples were evaluated for anti-VEGF protein levels using an MSD assay
  - **Perilymph:** Two months post-administration of AK-antiVEGF at 5X dose, anti-VEGF levels ranged from 67.0 to 694.9 ng/mL (mean: 406.8 ng/mL)
  - **Serum:** 36 out of 44 (82%) post-administration serum samples were below the limit of detection (LOD) for anti-VEGF protein for 1X and 5X doses
    - Anti-VEGF protein was detected in serum at reported biologically active levels in only one 5X dose animal, and only at Day 14 and Month 1; the Month 2 serum sample from this animal was below reported biologically active level
  - **CSF and flash-frozen tissues:** Anti-VEGF protein was not detected two or six months post-administration of AK-antiVEGF at 1X or 5X doses
  - NHP data support preliminary tolerability of localized anti-VEGF protein expression

Abbreviations: CSF = cerebrospinal fluid; MSD = meso-scale discovery; NHP = non-human primate(s); VEGF = vascular endothelial growth factor.

MSD LOD was 1.7 ng/mL and limit of quantification (LOQ) was 2.5 ng/mL

Expanded Exposure Evaluations Following AK-antiVEGF Administration in NHPs (AK-047)



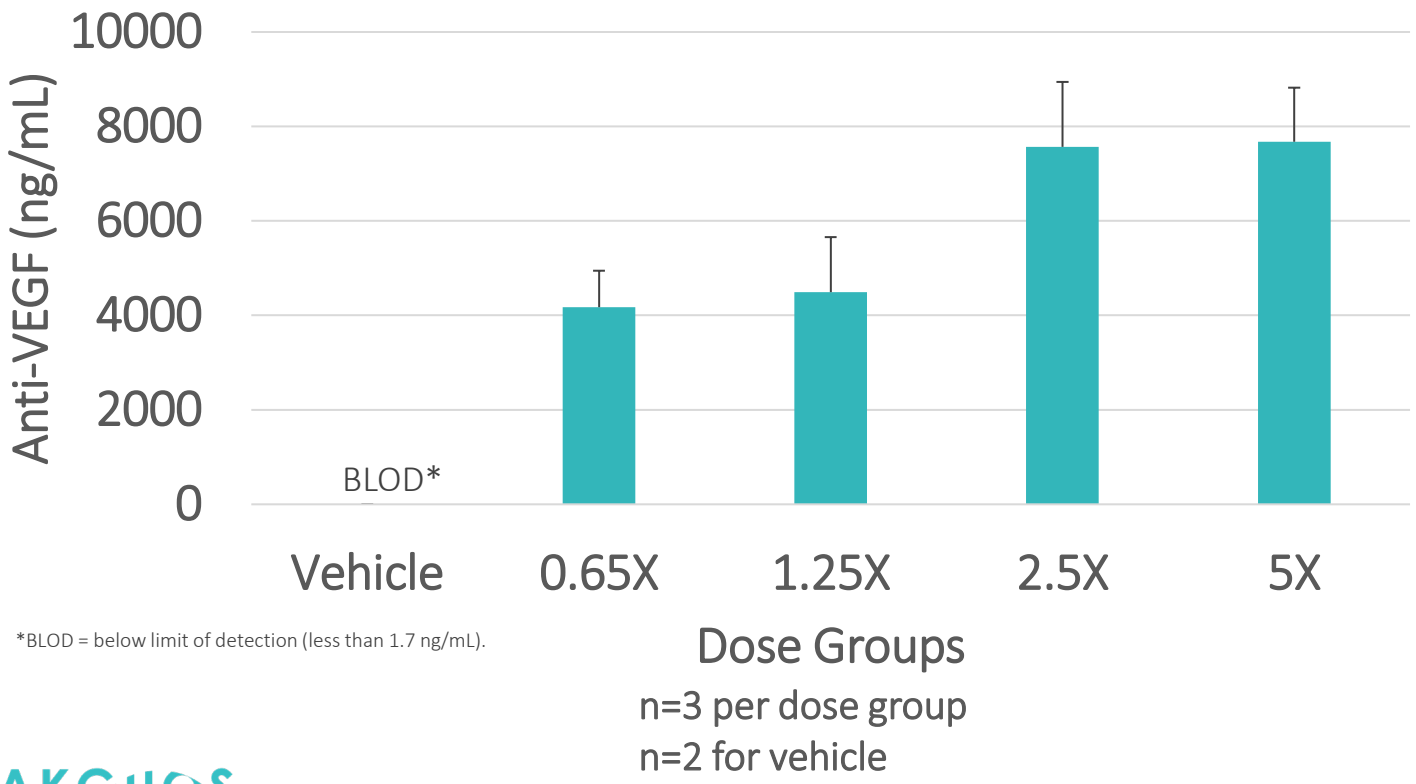


- Bilateral intracochlear administration of AK-antiVEGF (n=3 per group) or vehicle (n=2 per group)
  - Group 1: Vehicle control
  - Group 2: 0.65X dose
  - Group 3: 1.25X dose
  - Group 4: 2.5X dose
  - Group 5: 5X dose
- In vivo serum collection for MSD assay measurement of anti-VEGF levels
- Terminal fluid and unfixed (flash frozen) tissue collection for MSD assay measurement of anti-VEGF levels

Abbreviations: CSF = cerebrospinal fluid; NHP = non-human primate(s); MSD = meso-scale discovery; VEGF = vascular endothelial growth factor.

Intracochlear Administration of AK-antiVEGF Resulted in Local Expression of Anti-VEGF Protein in NHP Perilymph (AK-047)

Dose-dependent measurement of perilymph anti-VEGF levels two months post-administration



*BLOD = below limit of detection (less than 1.7 ng/mL).

Serum

Multiple timepoints (from Day 3 to Month 2):

No reported biologically active levels of anti-VEGF protein were detected in the serum of any NHPs, including control NHPs receiving vehicle

CSF and Flash-frozen Tissues

No quantifiable levels of anti-VEGF protein were detected in the CSF/tissues of any NHPs, including control NHPs receiving vehicle

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Abbreviations: CSF = cerebrospinal fluid; NHP = non-human primate(s); VEGF = vascular endothelial growth factor. Data cutoff: 22Oct2021

Computational Modeling Supports Reported Biologically Active Levels of Anti-VEGF at the Tumor Site

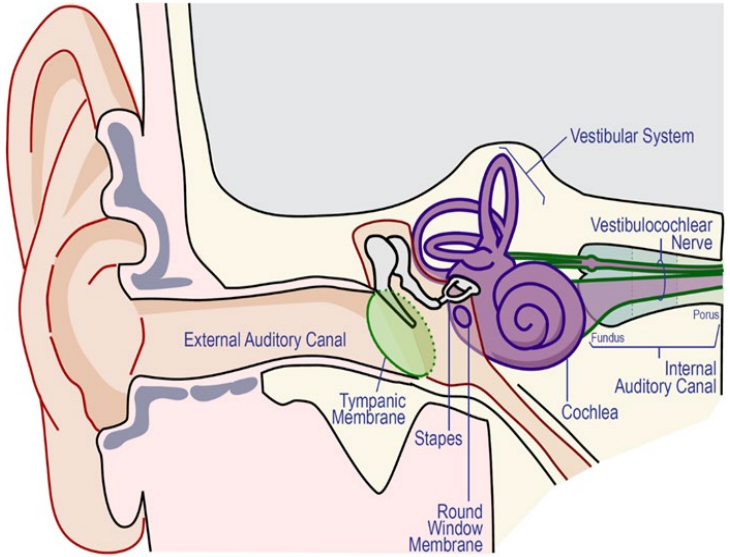
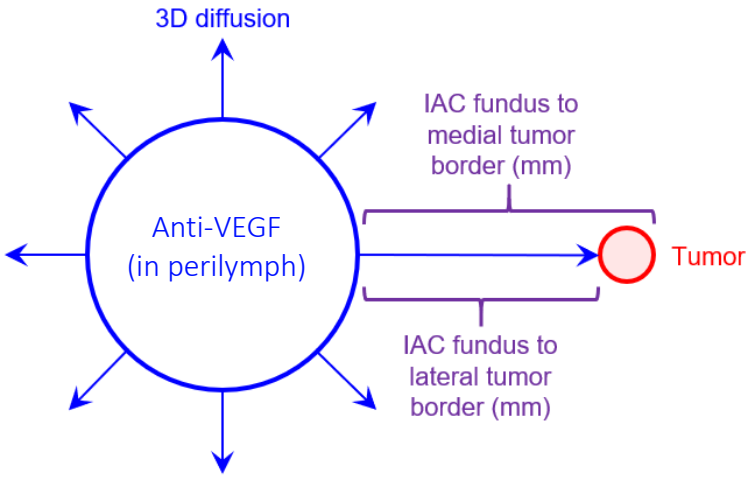


Image modified from:
https://commons.wikimedia.org/wiki/File:Anatomy_of_the_Human_Ear.svg



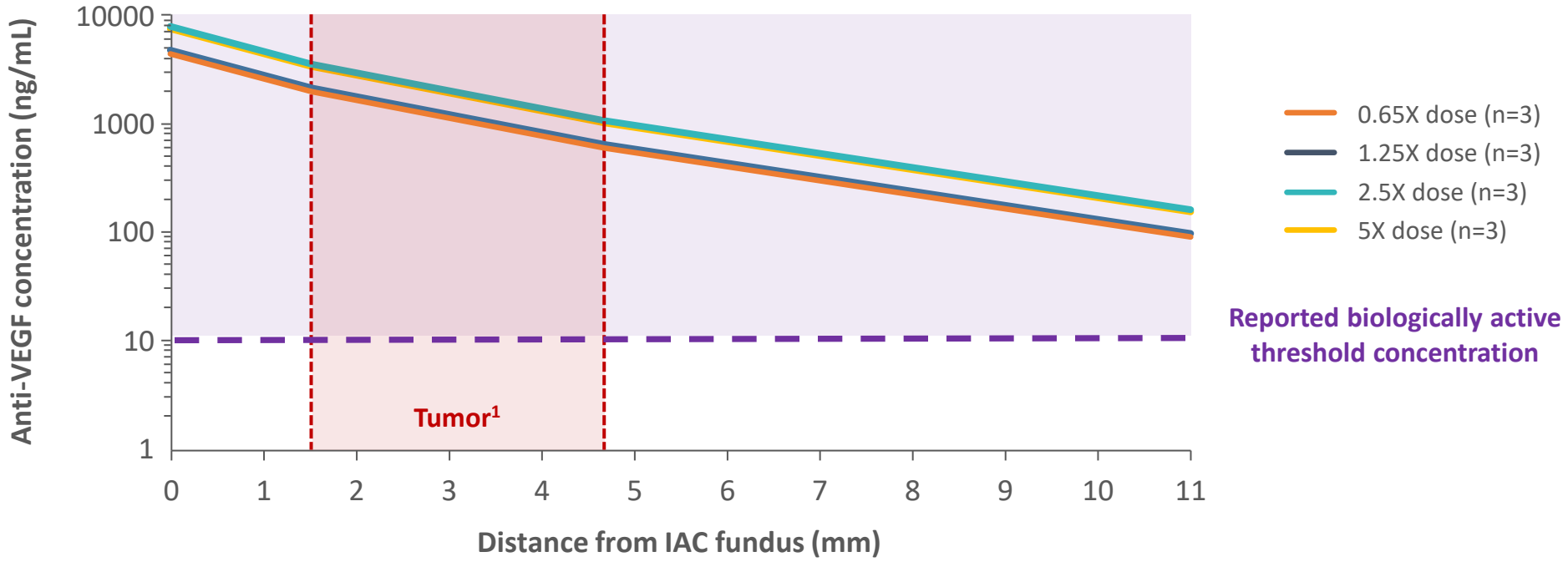
- Anti-VEGF secreted into the perilymph, or into the nerve interstitium, can reach the VS passively through simple diffusion, owing to lack of diffusion barriers from perilymph to the IAC
- Computational diffusional model estimates percent change in a (constant) concentration of anti-VEGF in perilymph as a function of distance to VS, using:
  1. The typical location of early VS from MRI data[1]
  2. A range of published diffusion coefficients for a representative anti-VEGF protein
  3. An estimated steady-state clearance parameter based on a representative anti-VEGF molecule half-life in vitreous following intraocular injection
  4. A conservative assumption of equal 360° diffusion in perilymph (i.e., assumes no anatomical constraints)
- Estimates of anti-VEGF concentration in the immediate vicinity of the VS in the IAC are well within the reported biologically active range

Abbreviations: IAC = internal auditory canal; MRI = magnetic resonance imaging; VS = vestibular schwannoma(s)

[1]Koen N, et al. Otolaryngol Head Neck Surg 2020; 162(2):211-214

Anti-VEGF Levels Are Modelled to Be Above Reported Biologically Active Concentration Across Range of Typical Early VS Locations

- Anti-VEGF levels in perilymph were measured in NHPs two months post-administration of AK-antiVEGF
- Computational modeling was used to assess approximate anti-VEGF levels at typical early VS locations[1]
- Using empirically determined anti-VEGF levels in the perilymph of NHP and conservative modeling assumptions, estimated anti-VEGF levels exceeded the biologically active threshold concentration within the range of early VS locations[1]



[1] Range of location of early VS from MRI data (Koen N, et al. *Otolaryngol Head Neck Surg.* 2020;162(2):211-214.)

Abbreviations: IAC = internal auditory canal; MRI = magnetic resonance imaging; NHP = non-human primate(s); VS = vestibular schwannoma.

Data cutoff: 22Oct2021

Conclusions

- There remains a high unmet need for alternative treatments for patients with VS
- Previously published clinical trial data support the potential for systemically administered VEGF inhibitors to be efficacious in treating *NF2*-related VS
    - However, associated toxicity can limit the chronic systemic administration of VEGF inhibitors as a viable treatment option for VS
- Local expression of anti-VEGF protein following intracochlear administration of AK-antiVEGF is robust and well tolerated in NHPs, an anatomically relevant model for evaluating delivery parameters
- Data from two studies across multiple doses demonstrate that systemic exposure to anti-VEGF protein is limited two months following intracochlear administration of AK-antiVEGF in NHPs
- Computational modelling supports the potential for diffusion of biologically active anti-VEGF protein levels to site of tumor
- Together, these data support the future clinical development of AK-antiVEGF for the treatment of VS
- IND submission for AK-antiVEGF is planned for 2022

Abbreviations: IND = investigational new drug; NF2 = neurofibromatosis type 2; NHP = non-human primate; MRI = magnetic resonance imaging; VEGF = vascular endothelial growth factor; VS = vestibular schwannoma(s).

Thank you!