Demonstration of Tolerability of a Novel Delivery Approach and Secreted Protein Expression Following Intracochlear Delivery of AK-antiVEGF (AAVAnc80-antiVEGF Vector) in Non-Human Primates



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Bellucci Symposia | June 3-4, 2021

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John Connelly is an employee of Akouos, Inc., and has received, and is receiving, compensation and equity from Akouos, Inc.

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### Vestibular Schwannoma

- Vestibular schwannomas (VS) are tumors resulting from neoplasia of the Schwann cells that ensheathe the vestibulocochlear nerve and is estimated to affect approximately 200,000 individuals in the United States and Europe
- VS may result in a variety of symptoms including hearing loss, tinnitus, headaches, and impaired balance; left untreated, VS can cause serious neurological problems, including facial paralysis, blindness, and brain damage severe enough to cause death
- Current standard of care:
  - Small or non-growing tumors may be followed by observation only
  - Surgical resection and/or radiation therapy are indicated for larger and/or progressive tumors
  - Surgical resection and radiation carry the risk of significant morbidity, including facial paralysis and hearing loss
- Systemic administration of VEGF inhibitors has shown promising results in a genetic form of VS (Neurofibromatosis Type 2 [NF2]) in clinical trials (Plotkin 2009 N Engl J Med; Plotkin 2012 Otol Neurotol; Lu 2019 J Neurooncol)
- Vascular endothelial growth factor (VEGF) is upregulated in vestibular schwannomas, including non-NF2 (sporadic) tumors (Koutsimpelas 2012 ORL J Otorhinolaryngol Relat Spec)



The most common area for VS occurrence is 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 (Koen 2020 Otolaryngol Head Neck Surg).

Image modified from: https://commons.wikimedia.org/wiki/File:Anatomy\_of\_the\_Human\_Ear.svg

## Rationale for AK-antiVEGF (AAVAnc80-antiVEGF) for the Potential Treatment of Vestibular Schwannoma

- Current standard of care (surgical resection and/or radiation) can have significant morbidity (e.g., facial paralysis and hearing loss)
- Vascular endothelial growth factor (VEGF) is upregulated in vestibular schwannomas, including non-NF2 (sporadic) tumors (Koutsimpelas 2012 ORL J Otorhinolaryngol Relat Spec)
- In clinical trial settings, repeated systemic administration of Avastin<sup>®</sup> (bevacizumab, a VEGF inhibitor) has been shown to decrease VS tumor size and improve hearing in NF2 patients (Plotkin 2009 N Engl J Med; Plotkin 2012 Otol Neurotol; Lu 2019 J Neurooncol)
- However, long-term systemic use of Avastin<sup>®</sup> is associated with toxicity
- Alternatively, a local low level of anti-VEGF protein could be efficacious, potentially reducing need for surgical resection and/or radiation

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Systemic Administration of VEGF Inhibitor Shows Promise in NF2-related Vestibular Schwannoma, but Toxicity Limits its Potential as a Viable **Treatment Option** 

Clinical Trial Data Demonstrate Ability of Systemic VEGF Inhibitor to Improve Hearing and Reduce Tumor Volume in Some Patients with Vestibular Schwannoma 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. Gregory Sorensen, M.D., Rakesh K. Jain, Ph.D., and Emmanuelle di Tomaso, Ph.D. Neuro-Oncology 12(1):14-18, 2010. NEURO-ONCOLOGY doi:10.1093/neuonc/nop010 Advance Access publication October 20, 2009 Bevacizumab induces regression of vestibular schwannomas in patients with neurofibromatosis type 2<sup>+</sup> 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. 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<sup>1</sup> · Vianney Gaillard<sup>2</sup> · Marc Baroncini<sup>3</sup> · Nicolas André<sup>4,5</sup> · Jean-Pierre Vannier<sup>6</sup> · Matthieu Vinchon<sup>3</sup> · Frederique Dubrulle<sup>2</sup> Jean-Paul Lejeune3 · Christophe Vincent7 · Véronique Nève8 · Héléne Sudour Bonnange1 Nicolas Xavier Bonne7 · Pierre Leblond

#### Meta-analysis: Clinical Trials of Efficacy and Safety of Bevacizumab in NF2 Patients

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

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Outcome	Pooled incidence (95% CI)	Outcome	Pooled incidence (95% CI)	
Radiographic resp	oonse	Complications		
Partial regressio	n 41% (31–51%)	Serious toxicity	15% (10-26%)	
Stable	47% (39–55%)	Hypertension	33% (20-45%)	
Progression	7% (1–15%)	Proteinuria	43% (23-64%)	
Hearing outcome		Amenorrhea	70% (51-87%)	
Improvement	20% (9–33%)	Other		
Stable	69% (51-85%)	Surgical interven-	11% (2-20%)	
Worsening	6% (1-15%)	tion		

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# AAVAnc80 Efficiently Transduces Multiple Cell Types in the Inner Ear

- Conducted nonclinical studies across three different species of non-human primates using 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 will produce and secrete anti-VEGF protein into the perilymph, a cochlear fluid in diffusional continuity with the interstitial and perineural spaces of the vestibulocochlear nerve where vestibular schwannoma tumors are located



GFP: green fluorescent protein; Myo7a: Myosin VII a; Tuj1: class III beta-tubulin All micrographs are from Study AK-007, with the exception of Satellite Glial Cells from Study AK-011

## Study AK-033: Preliminary Evaluation of Tolerability and Exposure of AK-antiVEGF in NHPs as Part of Overall Development Strategy

Attribute	Supporting Data
<b>Biological Plausibility</b>	Historical clinical trial data using Avastin <sup>®</sup> systemically in NF2 patients (Plotkin 2009 N Engl J Med; Plotkin 2012 Otol Neurotol; Lu 2019 J Neurooncol)
Tolerability	<ul> <li>Two- and six-month tolerability assessment in NHPs</li> <li>Auditory Brainstem Response</li> <li>Otic Histopathology</li> <li>Cytocochleograms</li> </ul>
Local Exposure Levels	Measurement of steady-state anti-VEGF levels in perilymph two months post- administration
Systemic Exposure	Measurement of anti-VEGF levels in serum (over 6 months) and terminal CSF (two- or six-months post-administration)
Clinical Translatability	Computational modeling calculating theoretical levels of anti-VEGF at typical site of early vestibular schwannoma (based on perilymph levels from AK-033)

Abbreviations: CSF: cerebrospinal fluid; NF2: neurofibromatosis type II; NHP: non-human primate

Additional work to further characterize tolerability and exposure is currently underway

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Study AK-033

### Preliminary Evaluation of Tolerability and Exposure of AK-antiVEGF in

Coiled cochlea











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



- Terminal fluid and unfixed (flash frozen) tissue analysis:
- Perilymph / CSF / serum: measurement of anti-VEGF levels
- Inner ears and vestibulocochlear nerves (cranial nerve VIII)
- Brain: auditory pathway
- Other major organs
- Bilateral auditory function tests (ABR); and

Terminal fluid and fixed-tissue analyses:

- Inner ears: otic histopathology (ear 1) or cytocochleogram analyses (contralateral ear)
- Brain: histopathology, with focus on auditory regions ٠
- CSF / serum: measurement of anti-VEGF levels

ABR: auditory brainstem response; kHz: kilohertz; CSF: cerebral spinal fluid



## Physiologic and Histologic Evaluations in NHPs Support Tolerability to AK-antiVEGF (Study AK-033)



Shifts in ABR thresholds (relative to baseline ABRs acquired in the same ear ~2 weeks prior to intracochlear administration) are shown for 1, 2, 3, and 6 months post-intracochlear administration of either vehicle or AAVAnc80 vector encoding anti-VEGF at doses of 1X vg/cochlea or 5X vg/cochlea. Group means (±SD) at each timepoint reflect bilateral measurements in each NHP on study.

- Mean ABR thresholds were similar to baseline thresholds across all frequencies following administration of AK-antiVEGF at two doses
- Minimal ABR threshold shifts were likely attributable to the surgical approach used in NHPs, which can affect sound conduction through the NHP middle ear; evaluations were comparable between animals receiving vehicle and either dose of AK-antiVEGF
- Cytocochleogram analyses revealed no signs of ototoxicity related to the test article
- No histopathology findings attributable to the test article

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• Initial NHP data supports preliminary tolerability of localized chronic anti-VEGF protein expression

## Intracochlear Administration of AK-antiVEGF Resulted in Local Expression of Anti-VEGF Protein in NHP Perilymph (Study AK-033)

Group	Animal ID	Test / Control Article (bilateral administration)	Dose (vg/cochlea )	In-life Duration (Months)	CSF Concentration (ng/mL)	<b>Left ear:</b> Perilymph antiVEGF Concentration (ng/mL)	<b>Right ear:</b> Perilymph antiVEGF Concentration (ng/mL)
6	6101	Vehicle		2	BLOD	BLOD	BLOD
7	7001	AK-antiVEGF	5X	2	BLOD	694.9	67.0
	7501	AK-antiVEGF	5X	2	BLOD	275.9	589.2
4	4001	Vehicle		6	BLOD	Perilymph samples were not collected per protocol; otic histopathology (ear 1) or cytocochleogram analyses (contralateral ear) were performed	
	5001	AK-antiVEGF	5X	6	BLOD		
5	5501	AK-antiVEGF	5X	6	BLOD		
	5502	AK-antiVEGF	5X	6	BLOD		

Abbreviations: BLOD = below limit of detection (less than 1.7 ng/mL); CSF = cerebrospinal fluid; mL = milliliters; ng = nanograms; NHP: non-human primate; vg = vector genomes

- Two months following intracochlear administration of AK-antiVEGF at 5X dose, anti-VEGF levels in perilymph ranged from 67.0 to 694.9 ng/mL (mean: 406.8 ng/mL)
- No anti-VEGF protein was detected in the perilymph of the vehicle-injected control NHP
- No anti-VEGF protein was detected in CSF, regardless of post-administration duration (2 or 6 months)
- Perilymph samples were tested using the meso scale discovery (MSD) assay; limit of detection (LOD) was 1.7 ng/mL and limit of quantification (LOQ) was 2.5 ng/mL

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



- Anti-VEGF secreted into the perilymph, or into the nerve interstitium, can reach the vestibular schwannoma (VS) passively through simple diffusion, owing to lack of diffusion barriers from perilymph to the internal auditory canal (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. Typical location of early VS from MRI data (Koen 2020 Otolaryngol Head Neck Surg)
  - 2. A range of published diffusion coefficients for a representative anti-VEGF molecule
  - 3. Estimated steady-state clearance parameter based on a representative anti-VEGF molecule half-life in vitreous following intraocular injection
  - 4. 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 internal auditory canal are well within the reported biologically active range

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### Levels of Anti-VEGF are Modelled to Be Above Biologically Active Threshold Concentration Across Range of Reported VS Locations

- Anti-VEGF levels in perilymph were measured in NHPs two months following intracochlear administration of AK-antiVEGF
- Computational modeling was used to assess approximate anti-VEGF levels at reported VS locations
- 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 reported range of early VS locations (Koen 2020 Otolaryngol Head Neck Surg)



## Anti-VEGF Protein Expression Largely Confined to Inner Ear (Study AK-033)

- NHPs on study were evaluated for circulating anti-VEGF levels in serum at Baseline, Day 14, Month 1, and Month 2; animals in the 6-month groups were also evaluated at Month 3 and Month 6
- 36 out of 44 (82%) post-administration serum samples were below the limit of detection (LOD) for anti-VEGF protein in both the 1X and 5X doses
- Anti-VEGF was detected in serum at biologically relevant levels in only one high-dose animal, and only at Day 14 and Month 1; the Month 2 serum sample from this animal was below biologically relevant levels
- Anti-VEGF was not detected in any CSF samples
- Anti-VEGF was not detected in any non-cochlear tissues evaluated, including liver, spleen, brainstem, auditory cortex, or mandibular lymph nodes
- Serum, CSF, and tissue samples were tested using the meso scale discovery (MSD) assay; LOD was 1.7 ng/mL and limit of quantification (LOQ) was 2.5 ng/mL

### Conclusions

- There remains a high unmet need for alternative treatments for patients with vestibular schwannoma (VS)
- Previous published clinical trial data (Plotkin 2009 N Engl J Med; Plotkin 2012 Otol Neurotol; Lu 2019 J Neurooncol) support the potential for VEGF inhibitors to be efficacious in treating VS when delivered systemically
  - However, associated toxicity limits the chronic systemic administration of VEGF inhibitors as a viable treatment option for VS
- Long-term, 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
- 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 filing for AK-antiVEGF is expected in 2022

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