The Adeno-associated Viral Anc80 (AAVAnc80) Vector: Precision Genetic Medicines to Address Hearing Loss

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Director, Anatomy & Physiology

AKOUOS

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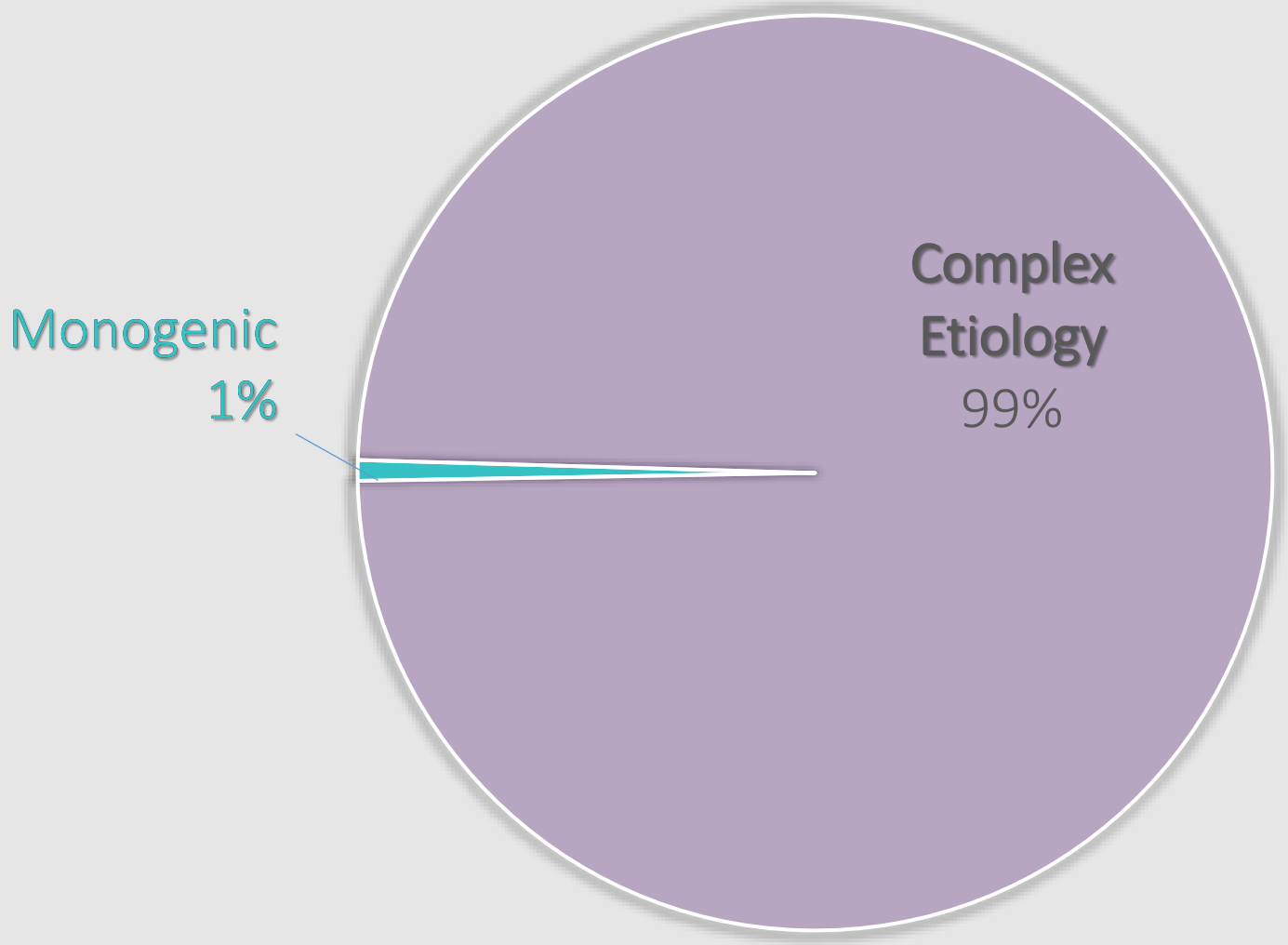
Disclosure

Within the last 12 months, I have had a financial arrangement and affiliation with a commercial interest related to the content of this symposium talk.

I receive a salary from and hold equity in Akouos, Inc.

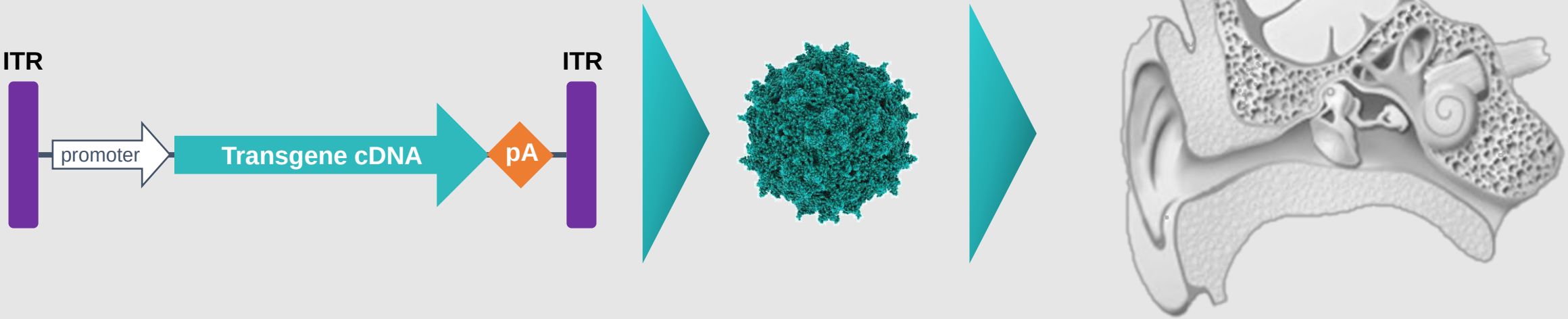
Who May Benefit from Cochlear Gene Therapy?



In vivo Gene Therapy Delivers Genetic Material to Target Cells

Direct delivery of a **functional gene copy** to the inner ear

Packaged into **Adeno-associated Viral (AAV) Vectors** for transport to nuclei of cochlear cells



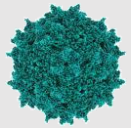
→ **Functional protein produced by cochlear cells** → **Improved hearing**

ITR = inverted terminal repeat; pA = polyadenylation sequence

Image sources: http://www.techillustration.co.uk/ ; https://www.abmgood.com/marketing/knowledge_base/Adeno_Associated_Virus_Introduction.php

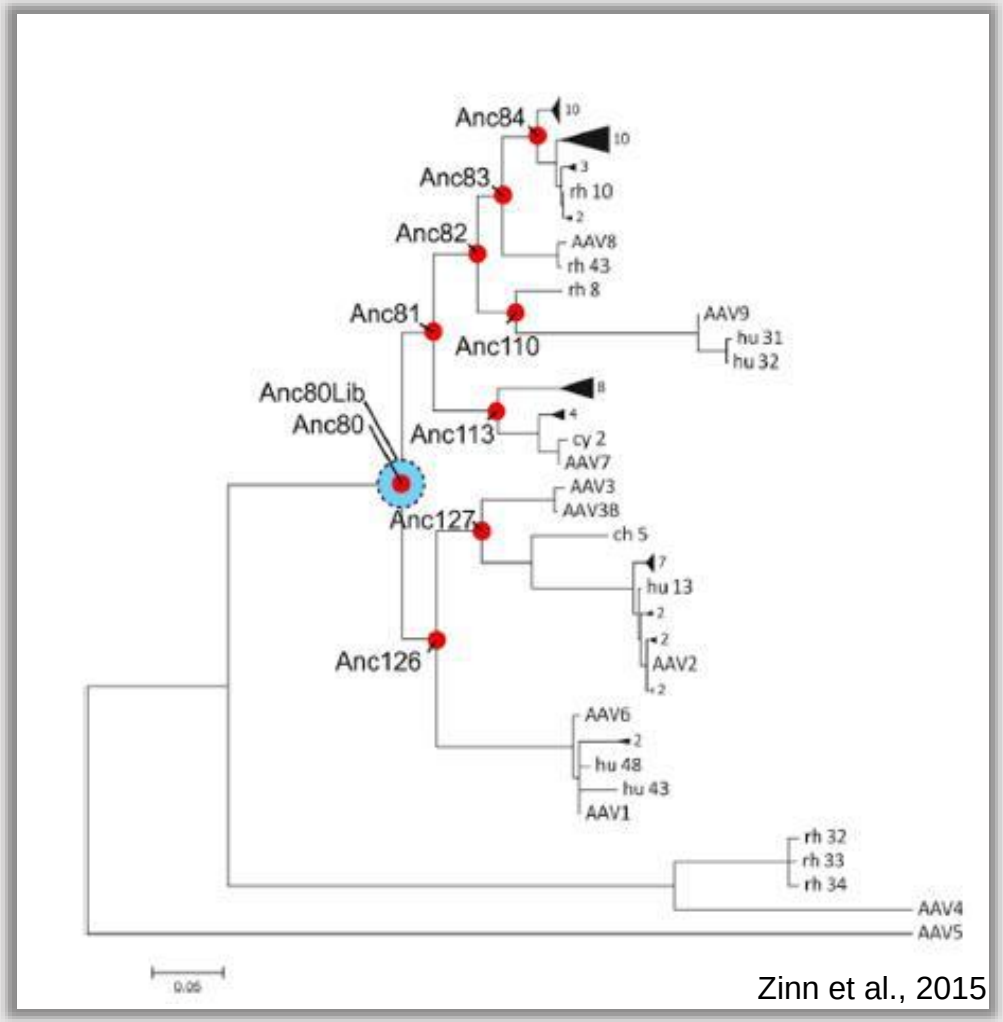
Viral Vectors for *in vivo* Cochlear Gene Therapy

Adeno-Associated Viral (AAV) Vectors



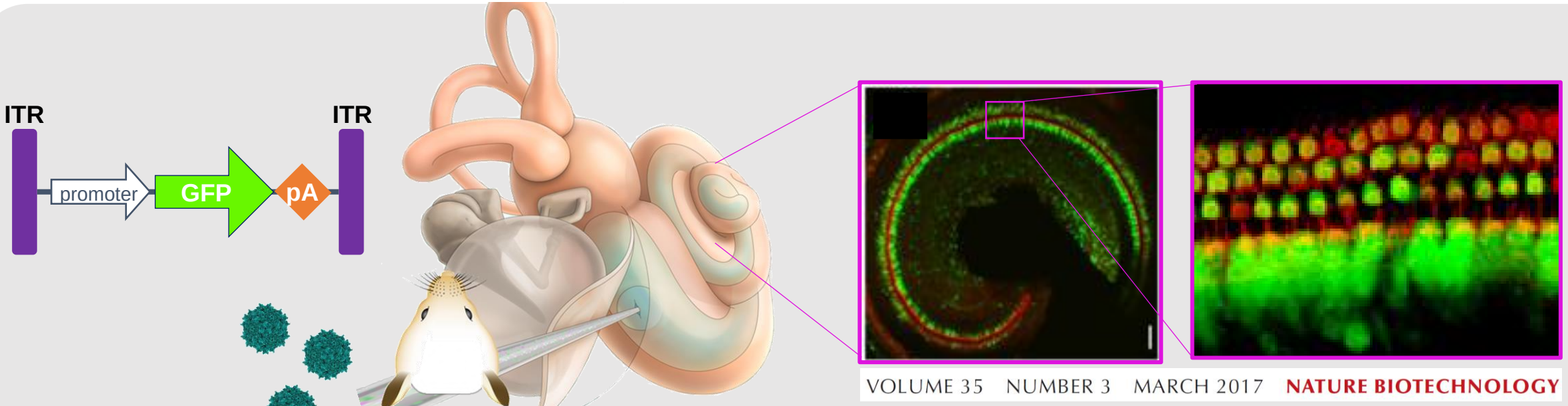
~25 nm

- Hundreds of people have been administered recombinant AAV in other therapeutic areas, with a good safety profile.
- Based on a non-pathogenic virus
- Persist episomally in the nucleus, with very low integration rates
- In non-dividing cells, a single administration could lead to *life-long transgene expression*
- Effectively transduce inner ear cells in animal models



Zinn et al., 2015

The AAVAnc80 Vector Transduces Cochlear Cells

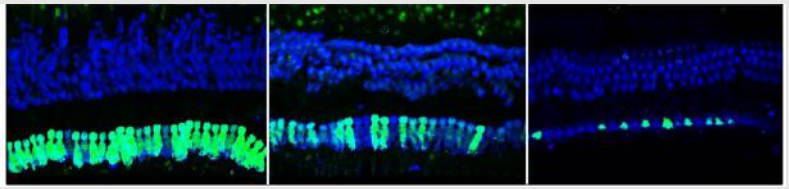


- Efficient transduction of inner and outer hair cells in mice
  - Landegger et al., 2017
- Partial recovery of auditory function in mouse models of deafness
  - Pan et al., 2017
- Will *in vivo* delivery of AAVAnc80 transduce the hair cells of NHP cochleae?

ITR = inverted terminal repeat; pA = polyadenylation sequence

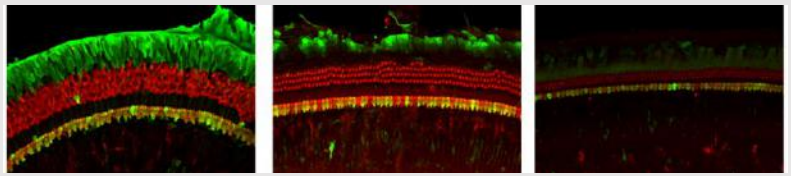
AAVAnc80 Transduction Evaluated in Three NHP Species

Rhesus (*Macaca mulatta*)
1-2 weeks post-injection (2wks shown)



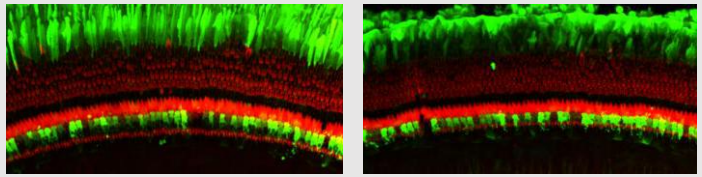
Andres-Mateos et al., 2019

Cynomolgus (*Macaca fascicularis*)
3 weeks post-injection



Poster 685
Monday

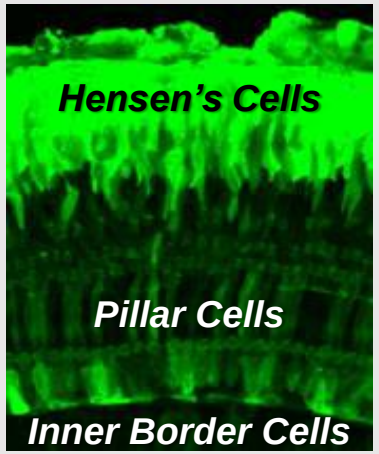
Baboon (*Papio anubis*)
3 weeks post-injection



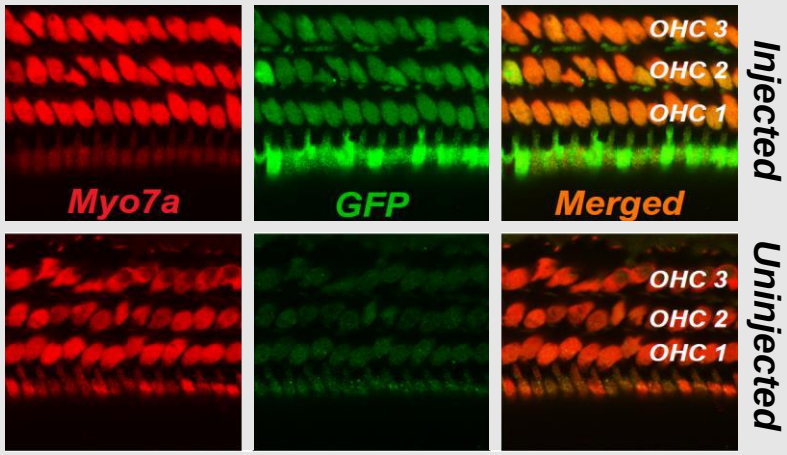
Poster 680
Monday

AAVAnc80 Transduces a Range of Inner Ear Cell Types

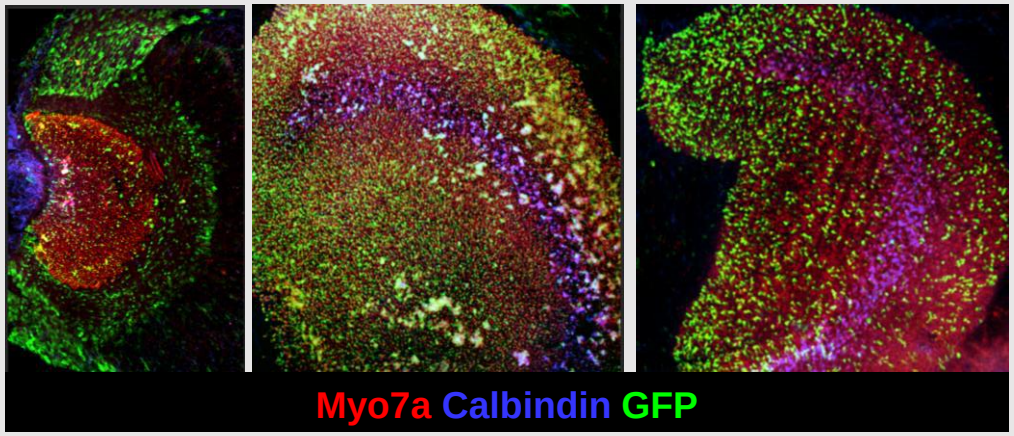
Supporting Cells



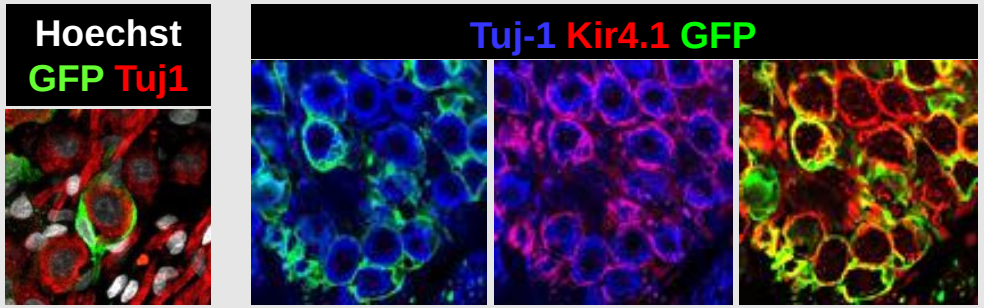
Outer Hair Cells



Vestibular Cells

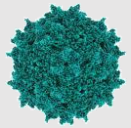


Satellite Glial Cells



Viral Vectors for *in vivo* Cochlear Gene Therapy

Adeno-Associated Viral (AAV) Vectors



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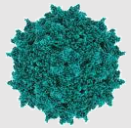
- **Hundreds of people have been administered recombinant AAV in other therapeutic areas, with a good safety profile.**
- **Based on a non-pathogenic virus**
- **Persist episomally in the nucleus, with very low integration rates**
- **In non-dividing cells, a single administration could lead to *life-long transgene expression***
- **Effectively transduce inner ear cells in animal models**
- **Complex manufacturing process and associated analytics**
- **Limited packaging capacity (~5 kB)**

- cDNA of some hearing-related genes exceeds packaging capacity
  - *OTOF, Myo7a, Atoh1, STRC*

Viral Vectors for *in vivo* Cochlear Gene Therapy

Adeno-Associated Viral (AAV) Vectors



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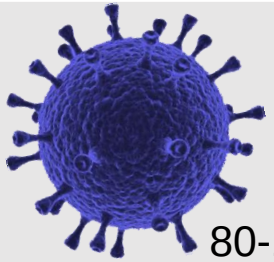
- Limited packaging capacity (~5 kB)

Lentiviral Vectors

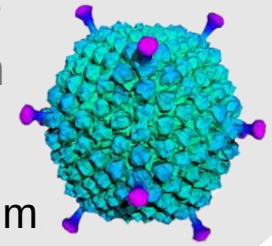


80-100 nm

- Larger packaging capacity (~9 kB)
- Integration into genome of host cell
  - Stable expression, even in dividing cells
  - Higher risk of insertional mutagenesis

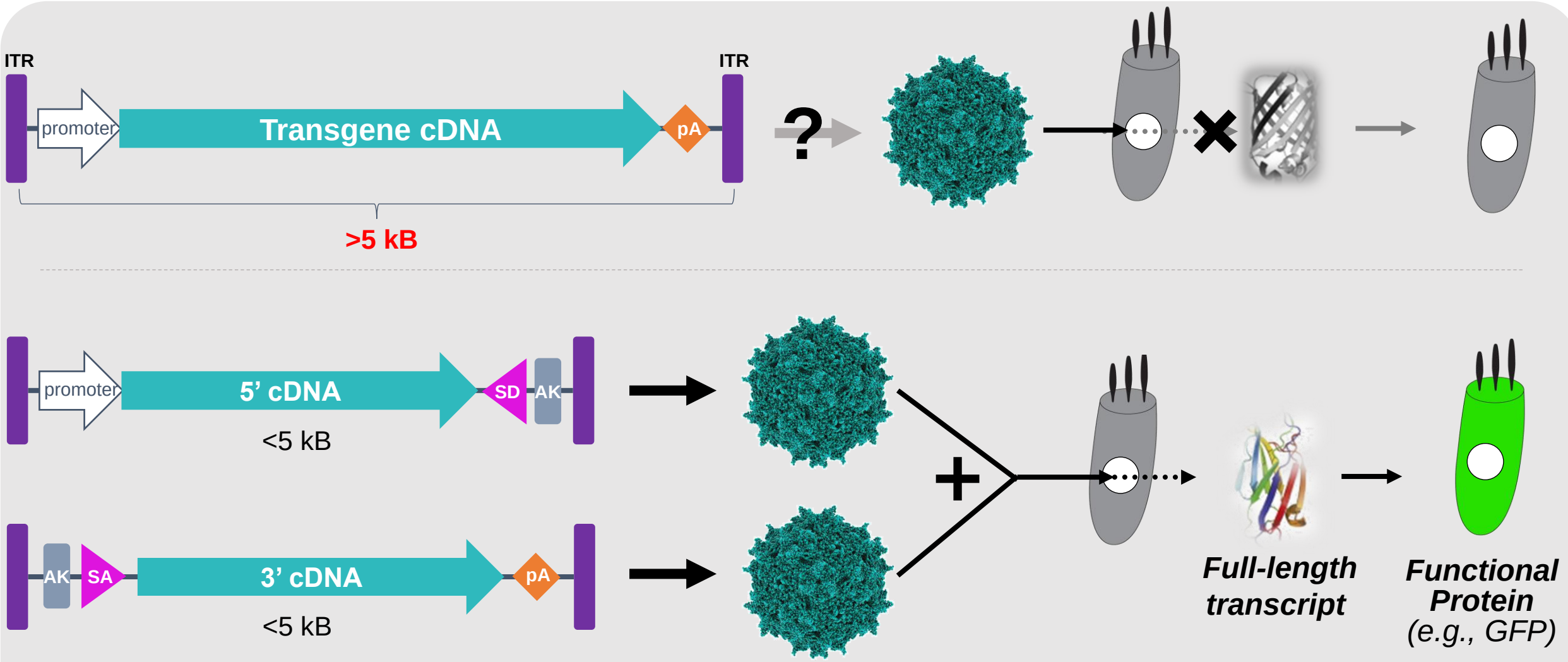
Adenoviral Vectors

- Larger packaging capacity (~8 kB)
- Simpler manufacturing process
- Transient transgene expression
- More immunogenic than AAV



80-100 nm

Dual-AAVAnc80 Approach for Larger Transgenes

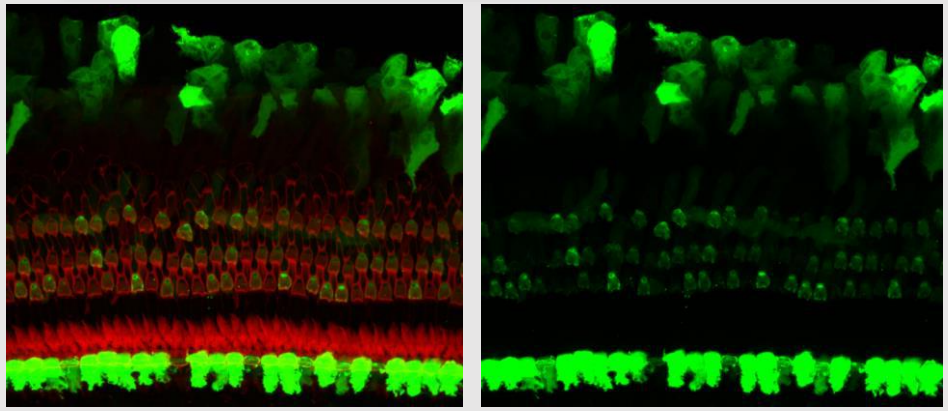


ITR = inverted terminal repeat; pA = polyadenylation sequence; SD = splice donor; SA = splice acceptor; AK =77 bp sequence from filamentous bacteriophage F1 (Trapani et al.,2014; 2015)

Efficient Transgene Expression in Inner Ear Cells of NHPs Following Administration of Dual AAVAnc80-eGFP

Poster 691

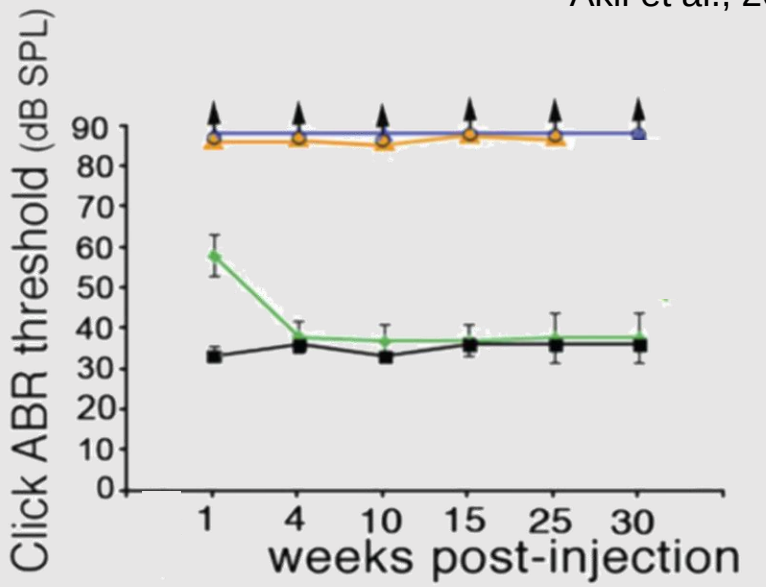
Cynomolgus (*Macaca fascicularis*)
3 weeks post-injection



Restoration of ABR Thresholds in *Otof*$^{-/-}$ Mice Receiving Dual AAV Vectors Encoding Otoferlin

Dual AAV delivering **mouse** *Otof*

Akil et al., 2019

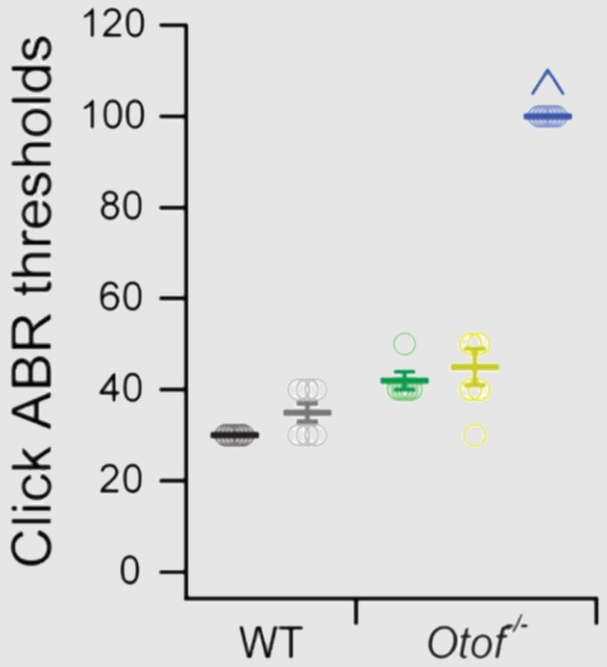


Wild-type
- Untreated (*n* = 8)

Otof$^{-/-}$
- + Dual AAV2-mOtof @ P10 (*n* = 8)
- +AAV2-mOtof N-terminal @ P10 (*n* = 3)
- Untreated (*n* = 6)

Dual **AAVAnc80** delivering **human** *OTOF*

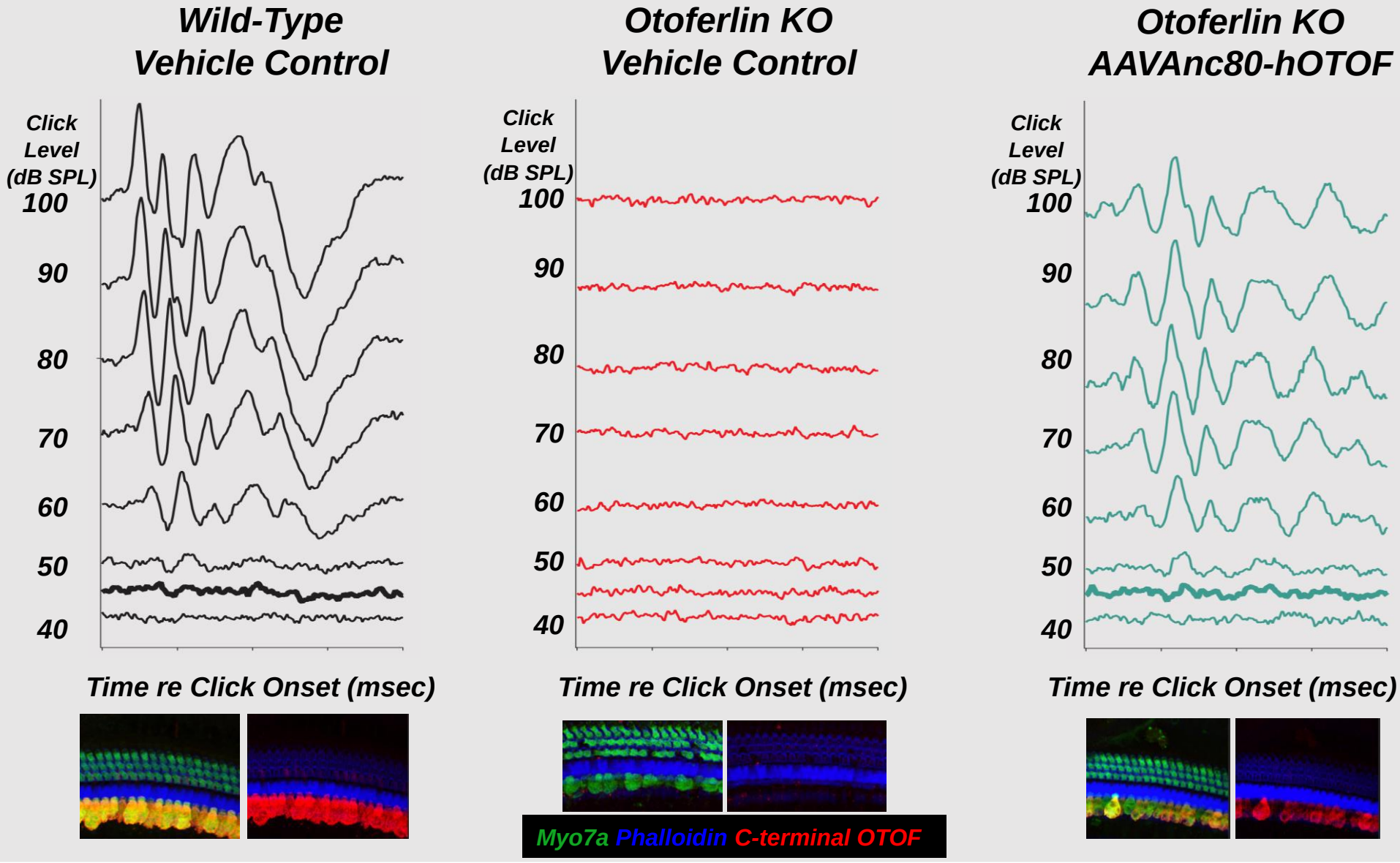
Data generated in collaboration with Ellen Reisinger



Wild-type
- Untreated (~4 wks, n=6)
- +AAVAnc80-eGFP (~3 wks, n=6)

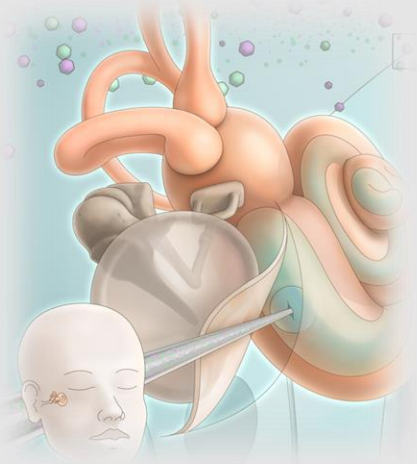
Otof$^{-/-}$
- +AAVAnc80-hOTOF-Dose 1 (~3 wks, n=5)
- +AAVAnc80-hOTOF-Dose 2 (~7 mo, n=5)
- Untreated (~4 wks, n=5)

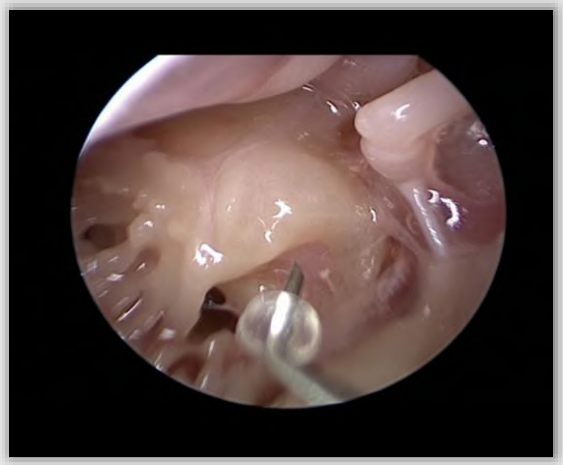
A One-Time Administration of Dual AAVAnc80-hOTOF Improves Cochlear Function in Mature Mice Lacking Otoferlin

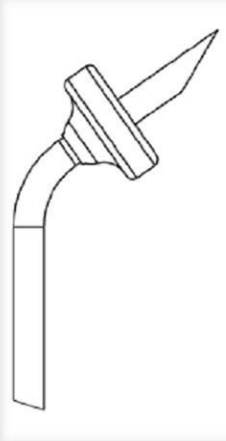


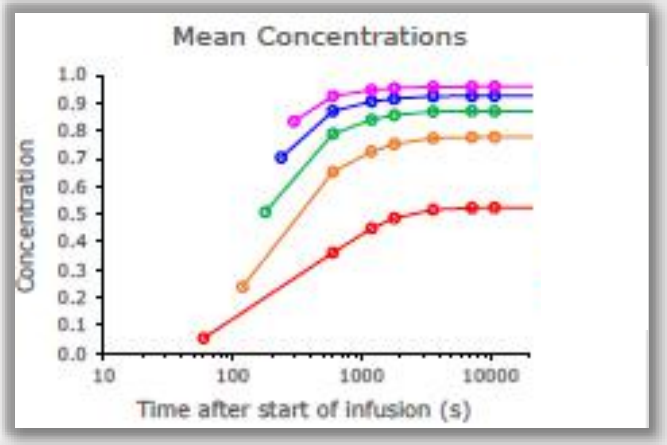
Intracochlear Delivery of AAVAnc80 in Humans will be Achieved via a Specialized Delivery Device

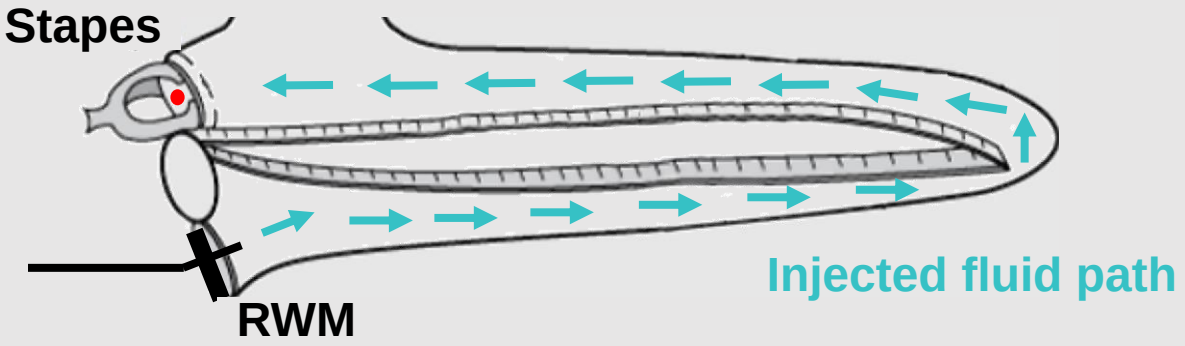
Minimally-Invasive Surgical Delivery











Summary

- AAV gene therapy is an FDA-approved treatment modality in different therapeutic areas and is a promising treatment approach for a wide range of hearing disorders.
  - The ability of AAVAnc80 to transduce a wide variety of cell types in the inner ear opens avenues to address many forms of genetic hearing loss.
  - AAVAnc80 can transduce the same inner ear cell types and achieve transgene expression in mice and multiple NHP species
- Dual AAVAnc80-hOTOF improves auditory function in young and mature mice, and expression of the otoferlin protein is limited to the IHCs despite AAVAnc80 transduction in other cell types.
- Delivery is a critical factor to translating preclinical studies into precision genetic medicines for individuals who could benefit from future therapies.