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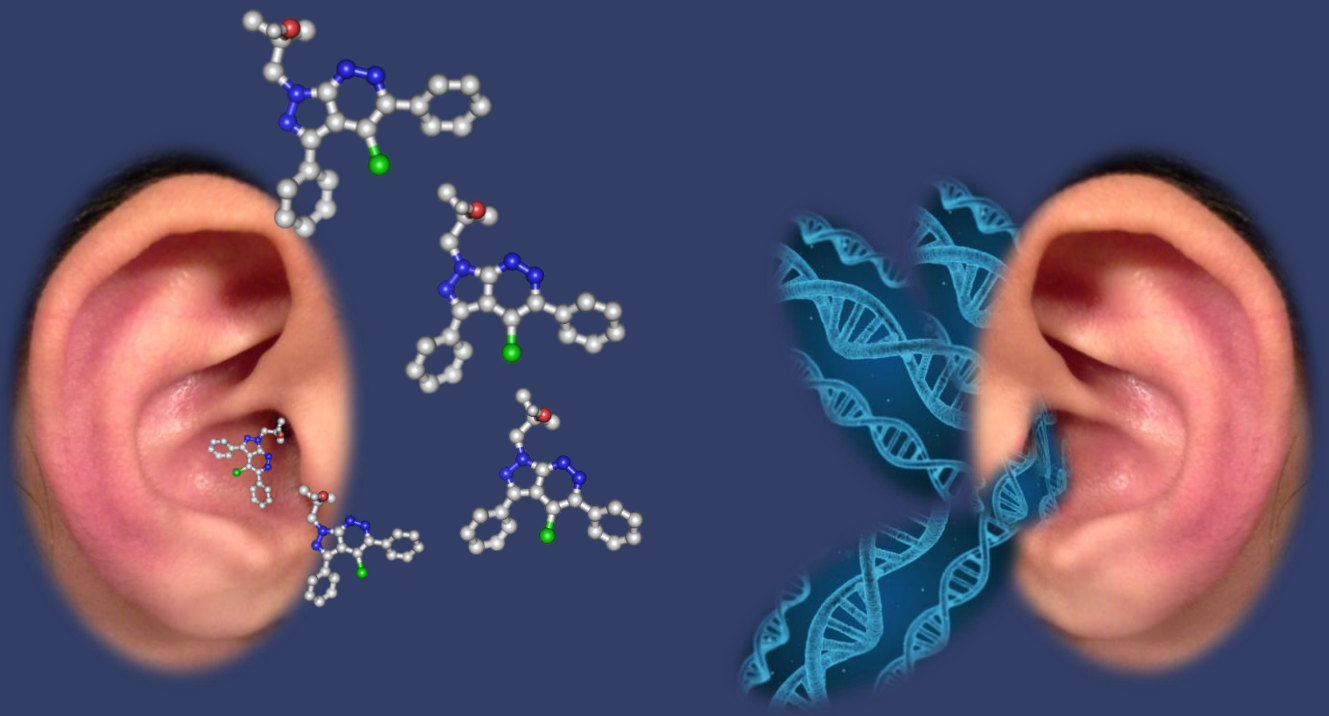


Small Molecule and Gene Therapy Approaches to Mitigate Hearing Loss in Usher Syndrome III

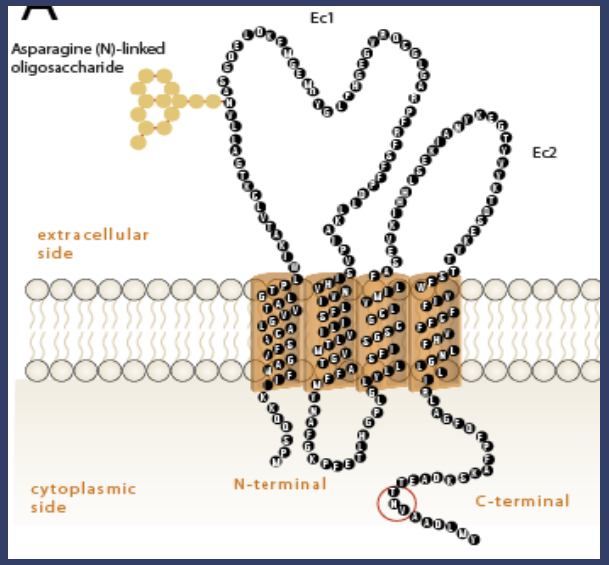
Background

- Post-lingual progressive hearing loss
- Variable presence of balance disorder
- Variable onset & severity of retinitis pigmentosa.

Usher Syndrome III (USH3)

- Mutation in the clarin-1 gene is linked to USH3*
- Clarin-1 protein (“CLRN1”), translated from the clarin-1 gene, is membrane proteins (symbol CLRN1)

Predicted structure



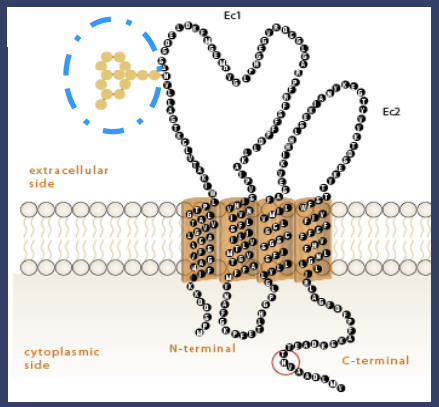
Tian et al., JBC 2009

* Joensuu et al *Am. J. Hum. Genet.* 69: 673–684, 2001.
*Adato et al *Eur. J. Hum. Genet.*, 10, 339-350, 2002.

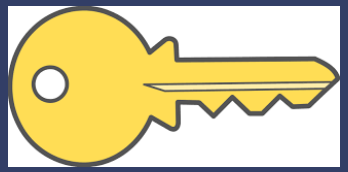
Two common mutations in Clarin-1

CLRN1

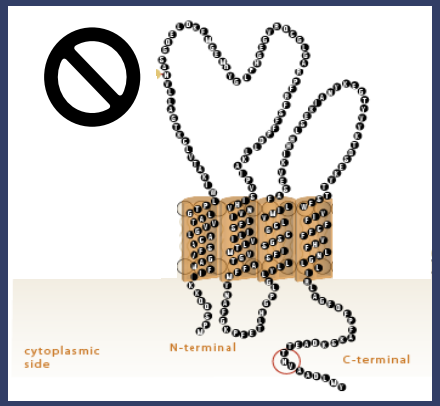
Sugar molecule



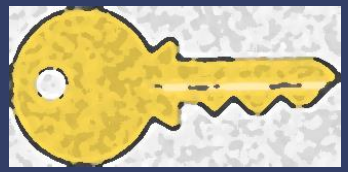
Normal

≈ 

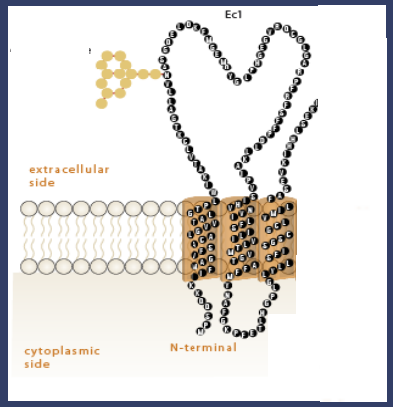
CLRN1^{N48K}



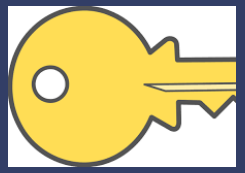
Unstable

≈ 

CLRN1^{Y176X}



"Null" effect

≈ 

General notes regarding my talk

1. Why hearing loss (HL) happens with CLRN1 Δ?
2. The small molecule drug we developed to mitigate hearing loss linked to $CLRN1^{N48K}$.
3. Gene therapy to preserve hearing loss in subjects with any Δ in CLRN1, including 'FIN major.'
4. Mouse models = mice carrying Δ in CLRN1 and showing hearing loss.
5. These mice do NOT develop eye disorder- we don't know why.

General notes regarding my talk

More than 10 years of research compressed into few slides

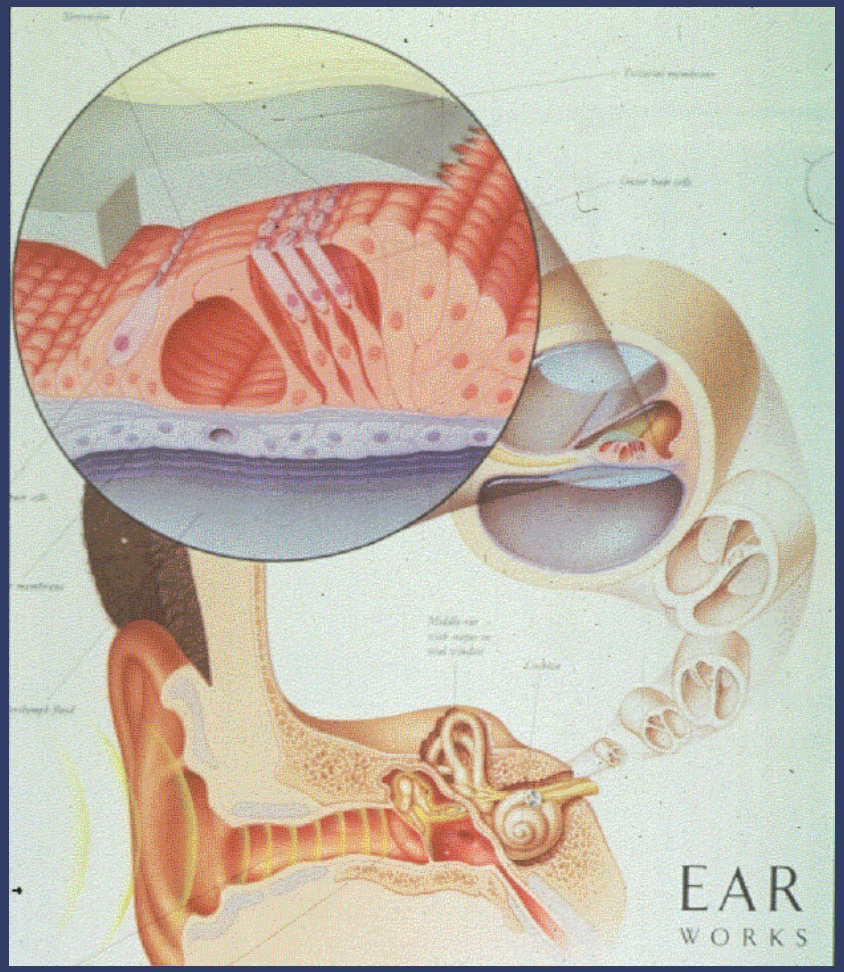
Details kept to a minimum

Please contact me at kna3@case.edu if you have question.

Human Ear Anatomy

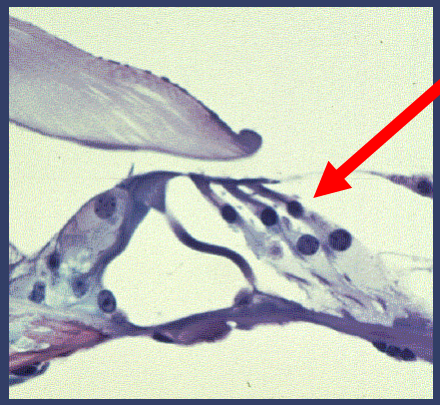
(Mouse ear is similar to human ear)

Human organ of Corti

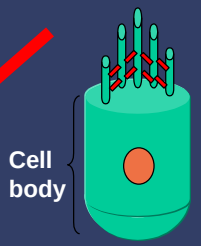


Picture from Discover Magazine

Mouse organ of Corti



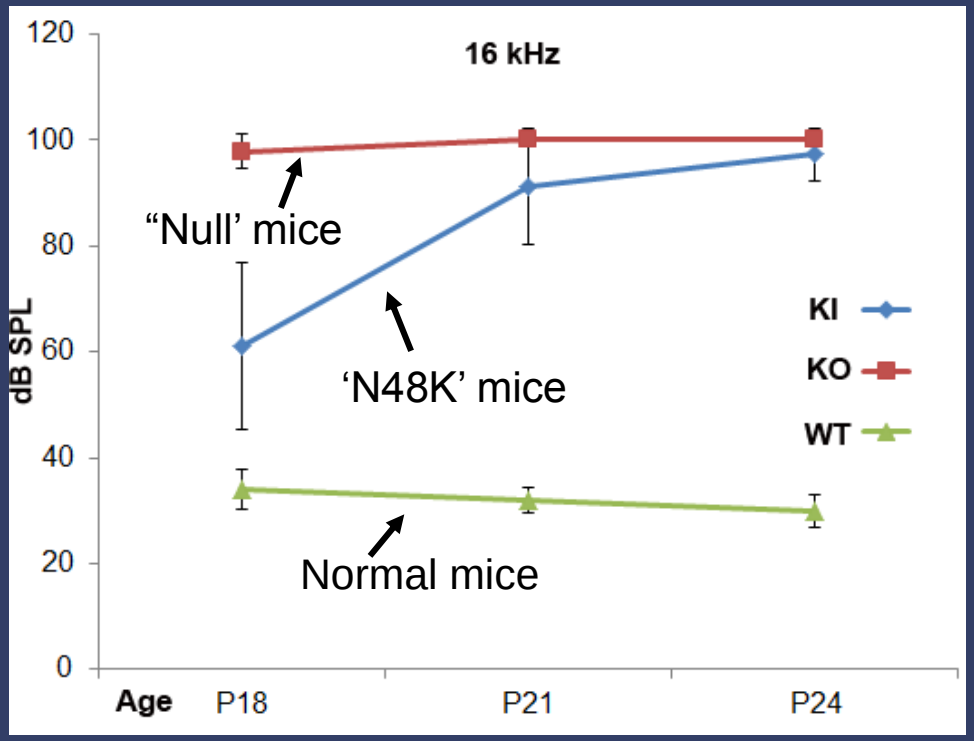
“Hair” Cell



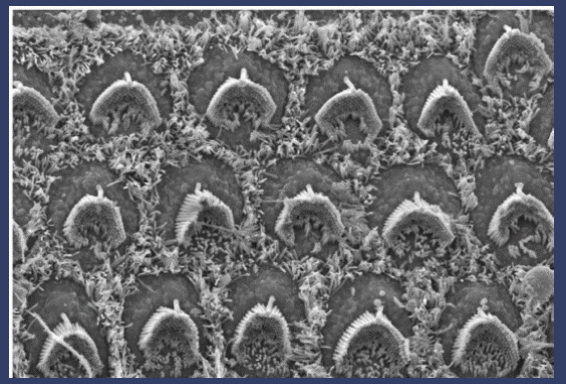
Hair cells convert sound vibrations into electrical signals → to the brain via the auditory nerve.

Why HL with CLRN1 Δ?
What do the mouse models tell us?

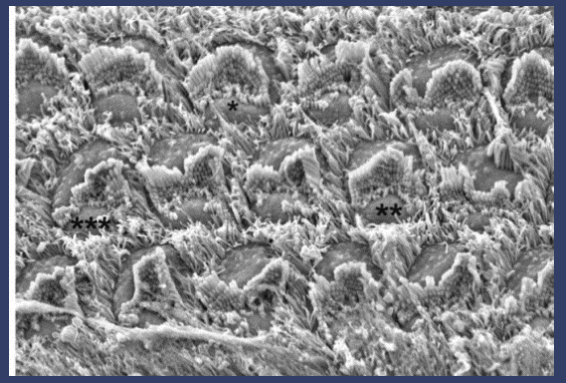
Hearing in mouse models of UsH3



Hair cells - normal mouse

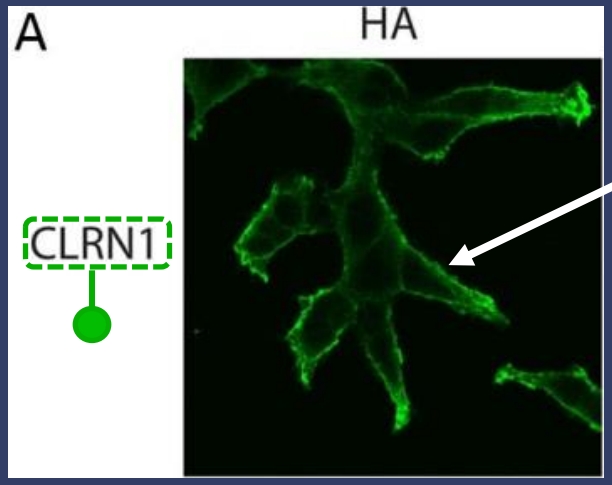


Hair cells - CLRN1 Δ mouse



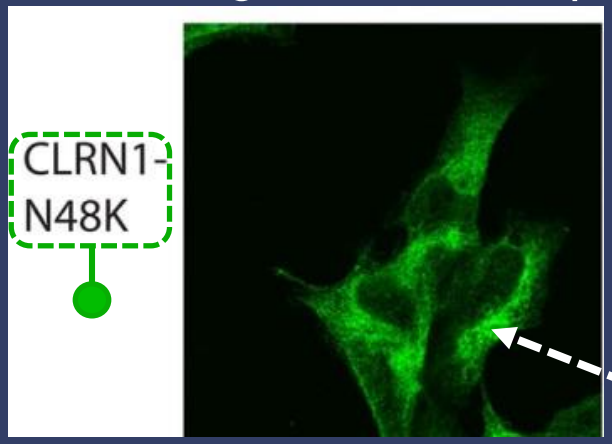
The impact of N48K mutation on CLRN1

Cells "making" CLRN1 protein



Normal CLRN1 protein goes to the cell membrane

Cells "making" CLRN1^{N48K} protein



1st, CLRN1^{N48K} protein can be seen in the cell ONLY if we add chemical agents to block protein degradation.

2nd,CLRN1^{N48K} is "stuck" inside the cell

Tian et al 2009; Geng et al., 2012

How to mitigate the CLRN1^{N48K} problem?

Hypothesis: Increasing the stability of CLRN1^{N48K} would help the mutant protein reach the proper site in the cell and rescue CLRN1-mediated function in the affected cells.

Proteasome inhibitors can increase CLRN1^{N48K} stability in cell culture, but we cannot use these as drugs for disorders like USH3 because it is toxic.

Small molecule therapy for CLRN1^{N48K} Δ

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 25 APRIL 2016 | DOI: 10.1038/NCHEMBIO.2069

A small molecule mitigates hearing loss in a mouse model of Usher syndrome III

Kumar N Alagramam[1–3]*, Suhasini R Gopal[1], Ruishuang Geng[1,9], Daniel H-C Chen[1], Ina Nemet[4], Richard Lee[4], Guilian Tian[4], Masaru Miyagi[5], Karine F Malagu[6], Christopher J Lock[6], William R K Esmieu[6], Andrew P Owens[6], Nicola A Lindsay[6,9], Krista Ouwehand[7], Faywell Albertus[7], David F Fischer[7], Roland W Bürli[6,9], Angus M MacLeod[6], William E Harte[8], Krzysztof Palczewski[4] & Yoshikazu Imanishi[4]*

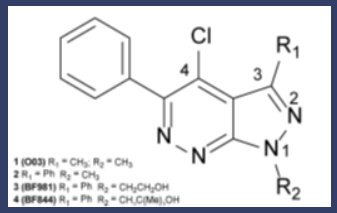
We = Team effort

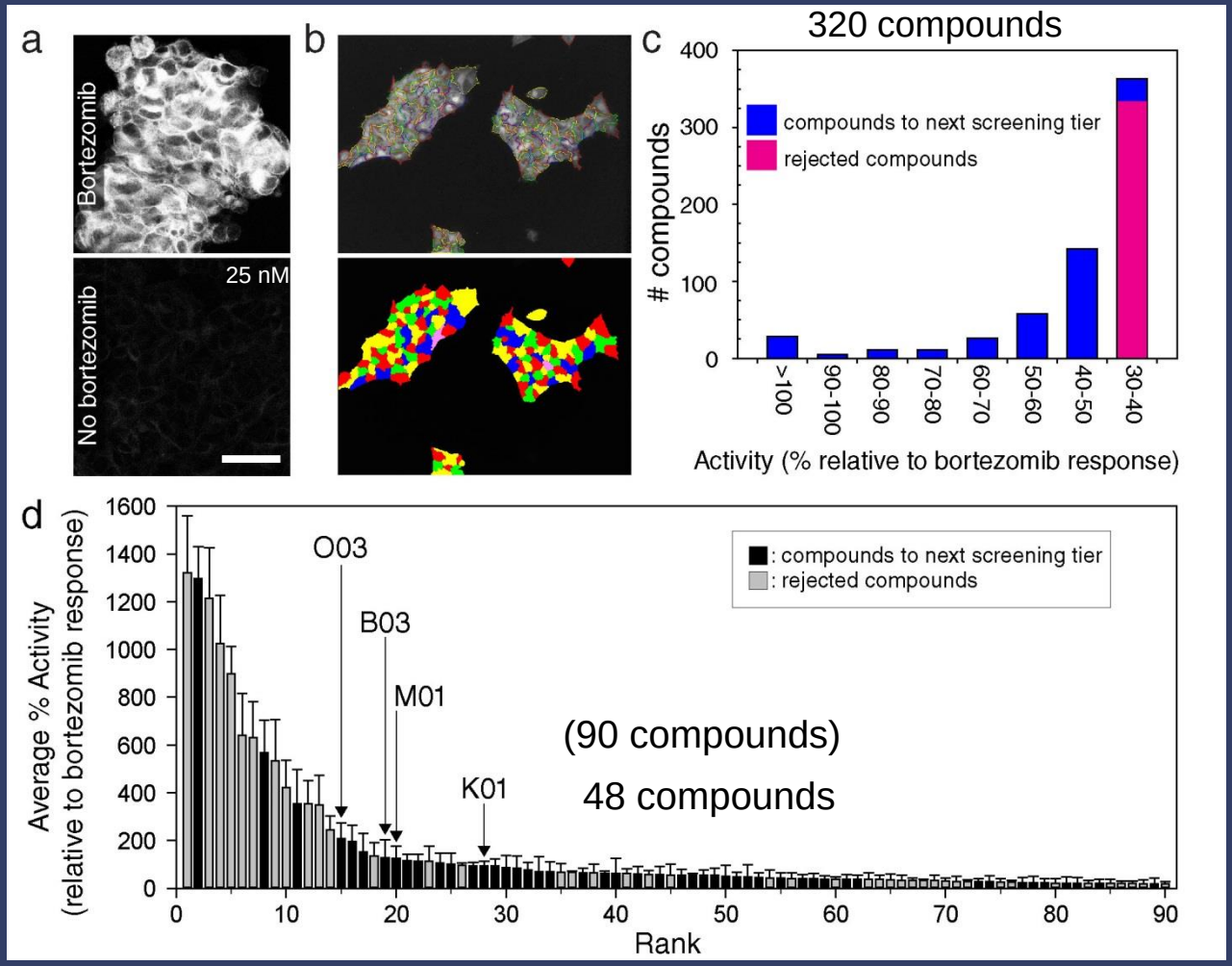
For author contributions, IP rights to the small molecule, etc., please refer to the published paper noted above.

50,000 small molecules screened

What's a small molecule?

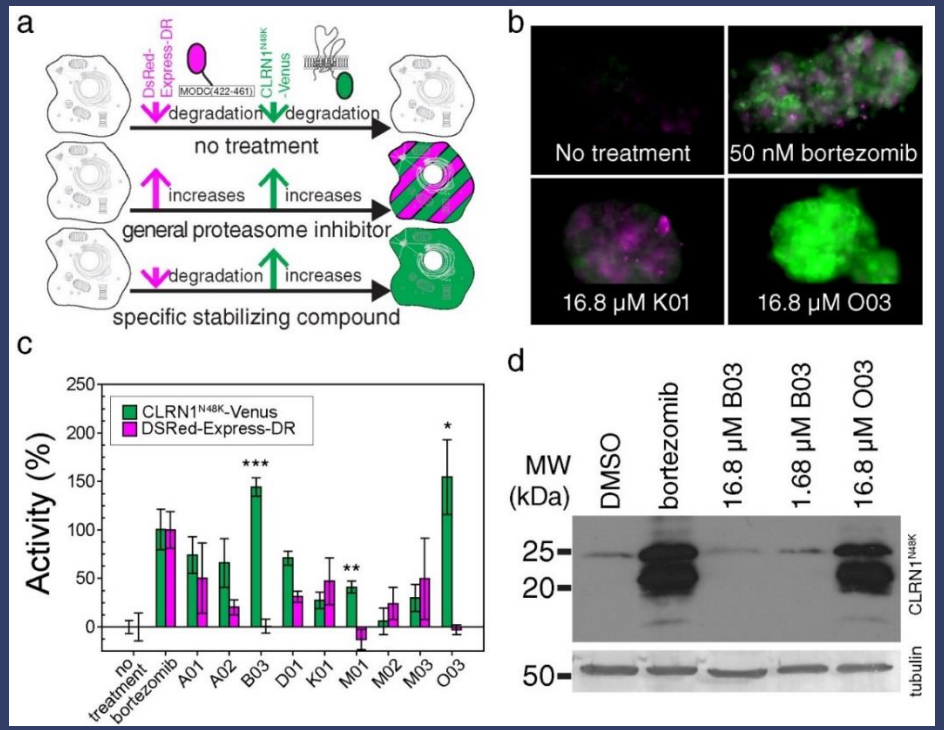
Example





Discovery of Lead Small Molecule - O03

- HTS of 50,000 compounds representing the chemical diversity space of pharmacologically relevant compounds were screened using a cell based immunofluorescence assay.
- A dual-reporter assay eliminates pan-proteasome inhibitors.
- From 50K → 1 lead compound "O03"

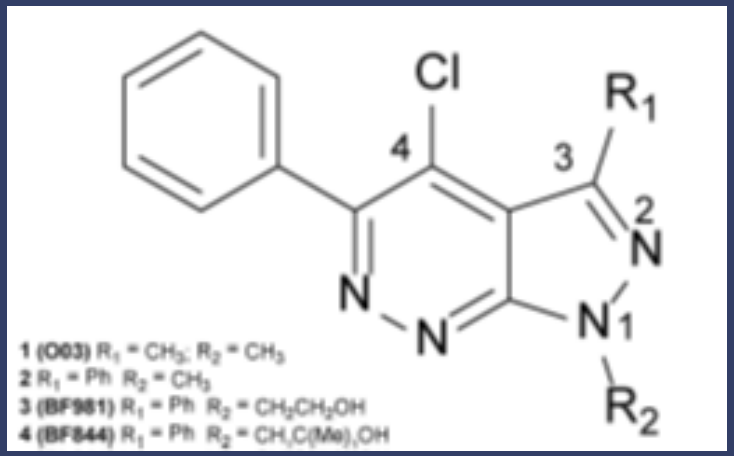


Oo3 stabilizes CLRN1^{N48K}, improving its chances of rendering clarin-1 mediated function in the cell

Optimized small molecule

O03 → **Lot of work** → BF844

~2 years



EC_{50} 2 μM

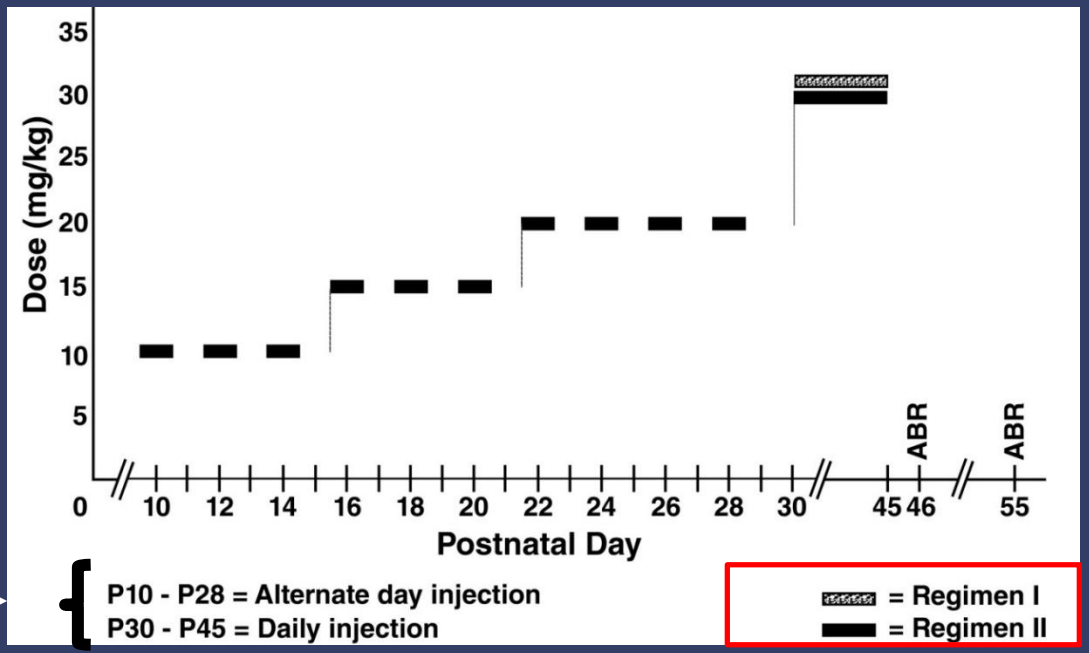
EC_{50} 0.34 μM

Testing the efficacy of BF844

BF844 was injected into CLRN1^{N48K} mice to test its ability to protect hearing protection in this model

We arrived at the doses based on *in vitro* and *in vivo* testing

Drug tested on young mice

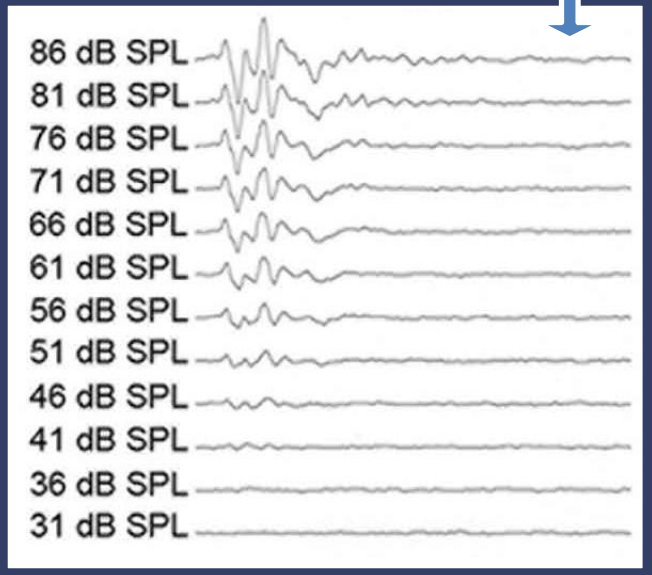


Hearing test used: Auditory Brainstem Response

Sound → Ear → auditory nerve → brain(stem) →

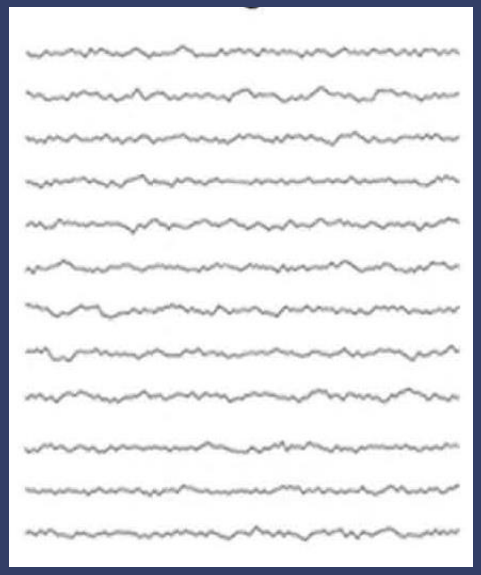
Waveform ← Recorder

Normal mouse



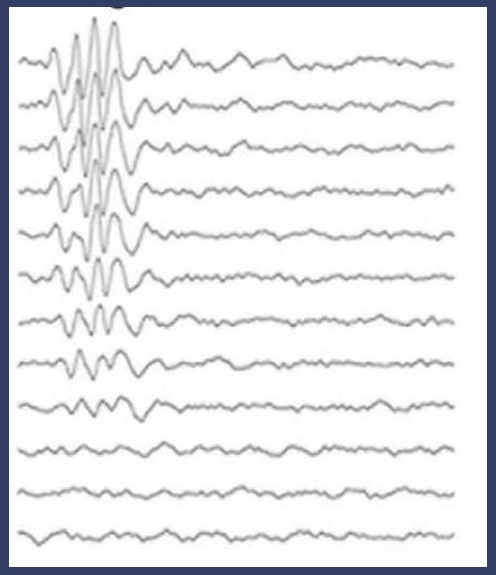
100 days old

USH3 mouse



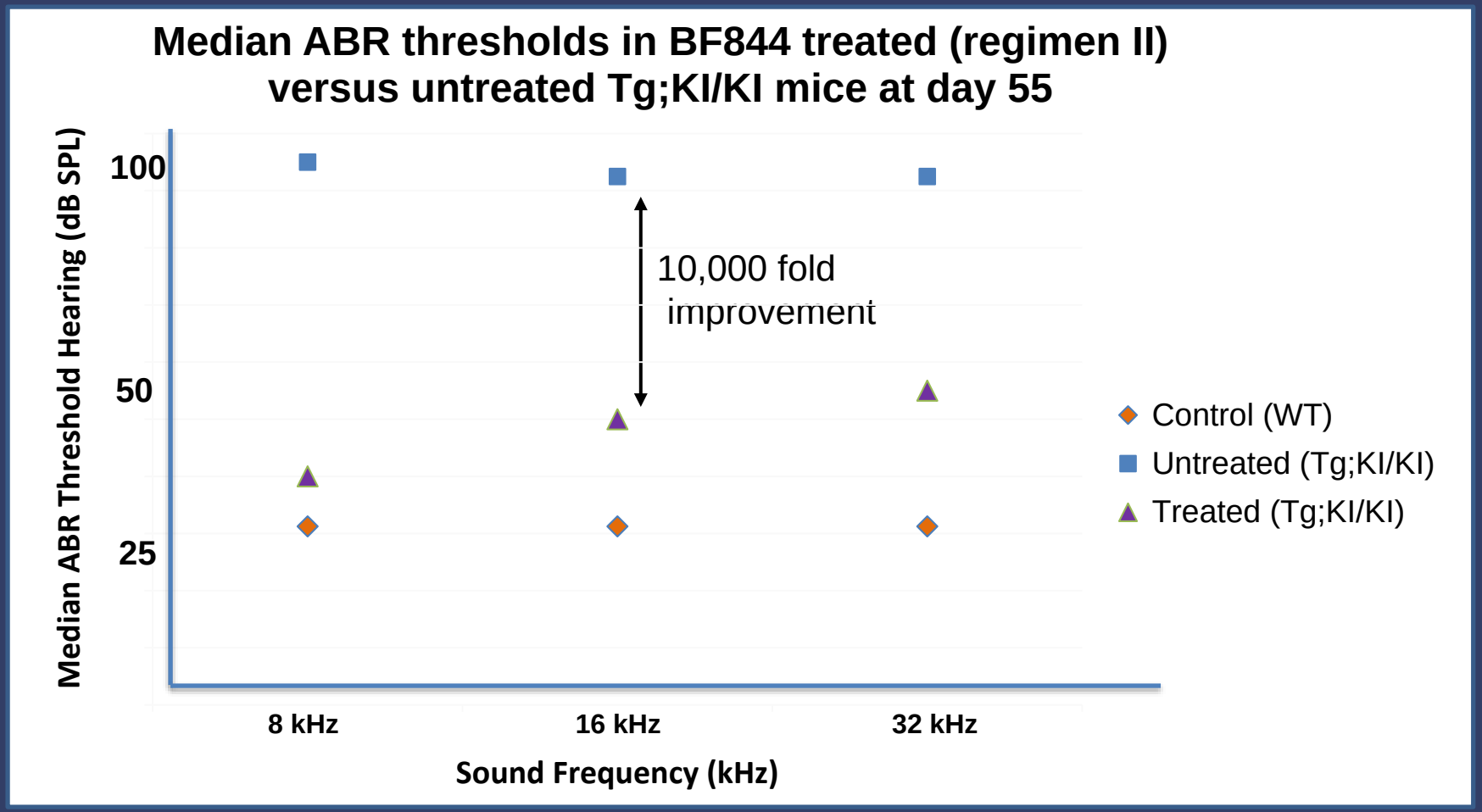
100 days old

Treated USH3 mouse



100 days old

BF844 mitigates HL in CLRN1^{N48K} Mice



Hearing improved in treated mice by 10,000 fold!

Small Molecule BF844

- 1st targeted therapy for an Usher Δ
- Preserves hearing in CLRN1^{N48K} mouse
- Since the CLRN1^{N48K} Δ causes both hearing and vision loss, BF844 administered systemically could in principle prevent both sensory deficiencies in patients with USH3
- In principle, the BF844 would have to be taken regularly, because sensory deficit in USH3 patients is a chromic disorder.

Current status?

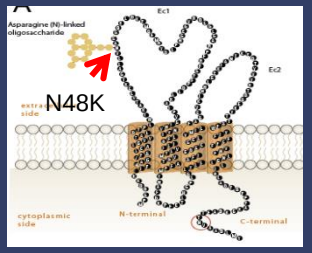
Now, we are here

- How old can we go?
- How “long” can we go?
- Long term?

Clinical trials

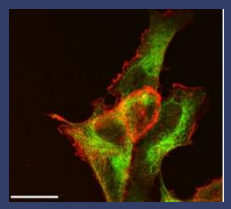
Preclinical studies

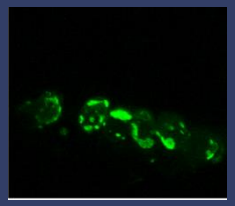
CLRN1-N48K Subjects

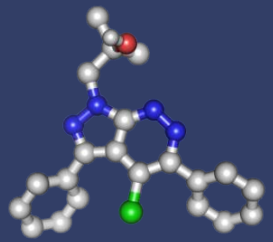


~10 years Of Work!

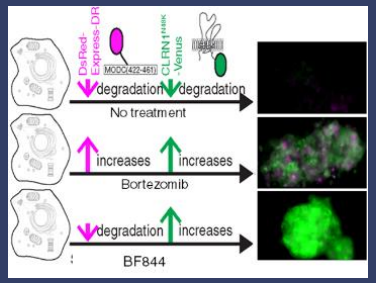
Blocks PHL





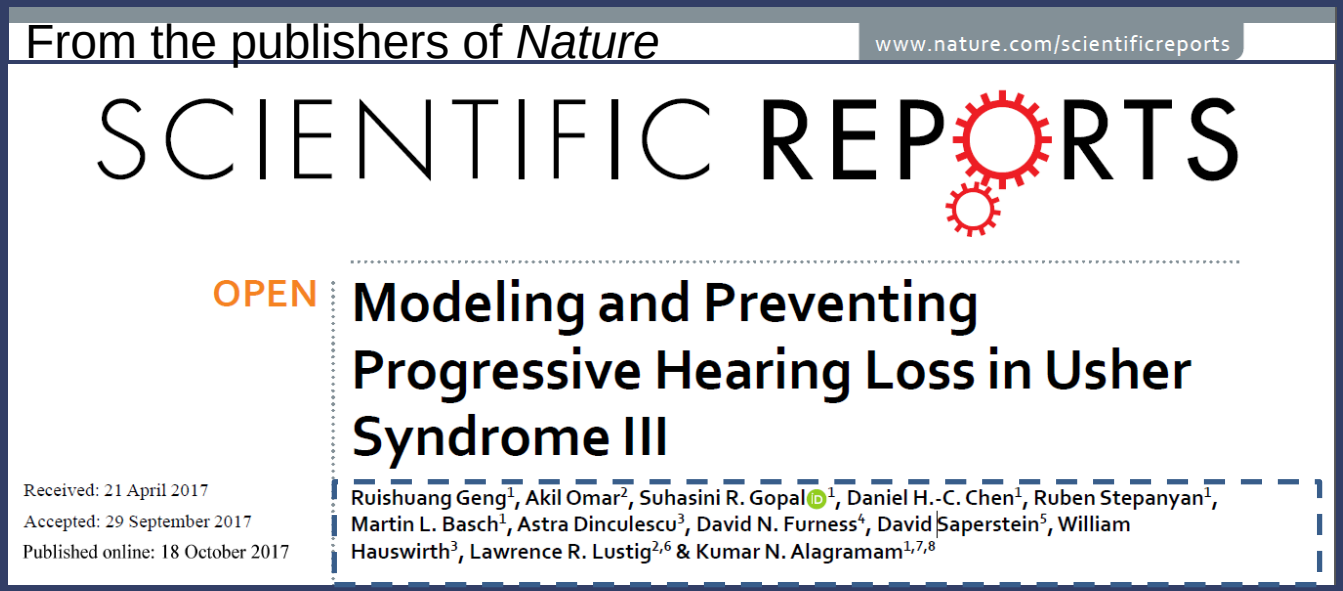


BF844



Part II of my talk: Exciting Development!

Gene therapy (**GT**) tested in USH3 mouse model; paper published 18th October, 2017!



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SCIENTIFIC REPORTS

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Modeling and Preventing Progressive Hearing Loss in Usher Syndrome III

Received: 21 April 2017
Accepted: 29 September 2017
Published online: 18 October 2017

Ruishuang Geng[1], Akil Omar[2], Suhasini R. Gopal[1], Daniel H.-C. Chen[1], Ruben Stepanyan[1], Martin L. Basch[1], Astra Dinculescu[3], David N. Furness[4], David Saperstein[5], William Hauswirth[3], Lawrence R. Lustig[2,6] & Kumar N. Alagramam[1,7,8]

We = Team effort

For details regarding author contributions, IP rights, etc., please refer to the published paper noted above.

Part II: Gene therapy (GT) approach

- GT: Normal copy of a gene is transplanted into Δ cells of the target organ to correct the genetic disorder.
- Why GT for USH3?
- BF844 won't work for all mutation in USH3
  Example, CLRN1^{Y176X} ≈ no protein made;
  GT will enable the synthesize CLRN1 protein
- GT can be used to treat any mutation in CLRN1
- So, why did we bother developing BF844 for CLRN1^{N48K} patients?

Part II: Gene therapy (GT) approach

- In case of CLRN1^{N48K} a ‘functional’ protein is made, but it needs ‘*help to keep up*’, to deliver CLRN1-mediated function; BF844 ‘assists’ the Δ protein to do so.
- BF844 can reach both eyes and both ears and treat all ‘4’ sensory organs at the same time.
- GT has to be done 1 eye and 1 ear at a time.

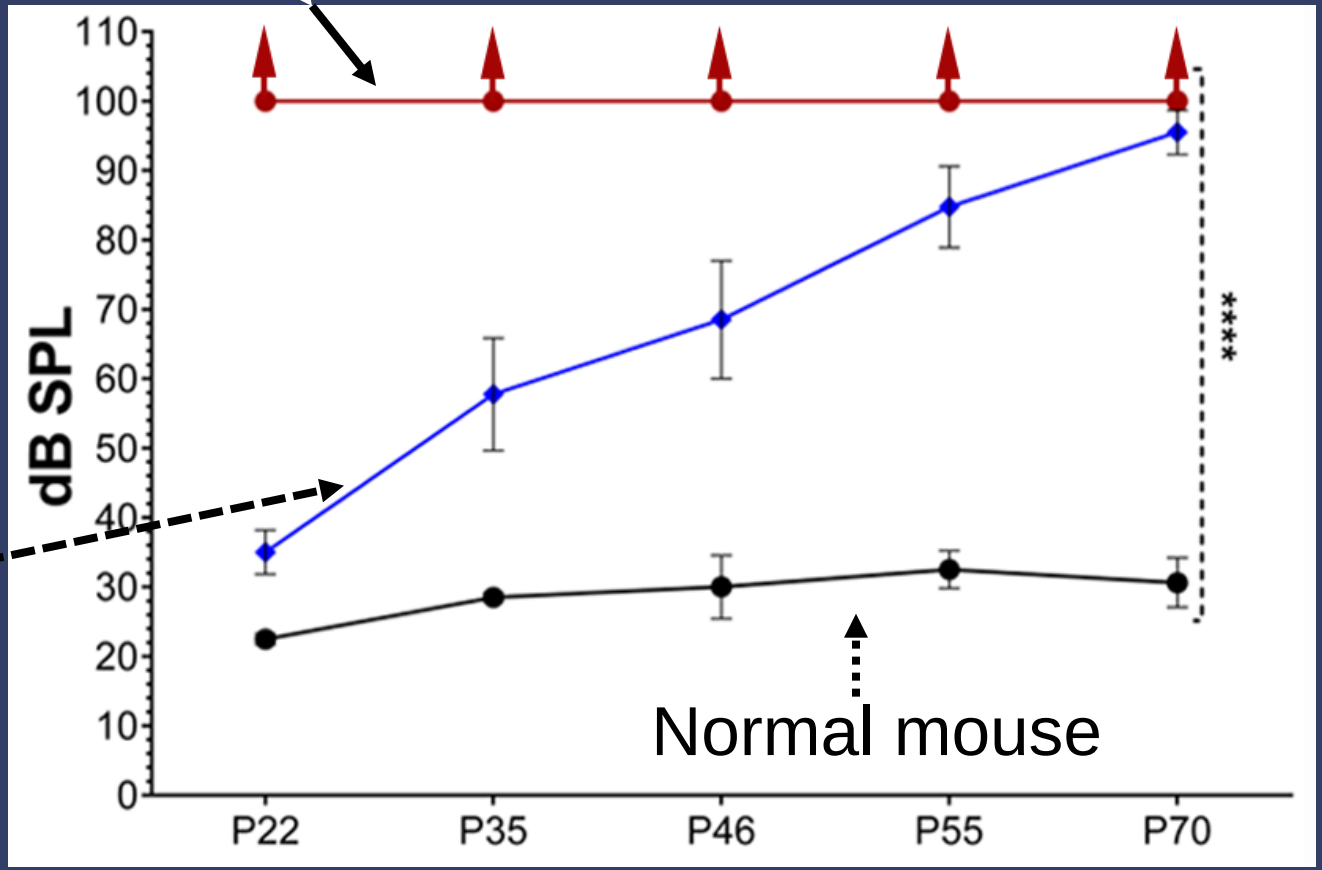
“New & Improved” Mouse Model for HL in USH3

Old model

--Early onset profound HL

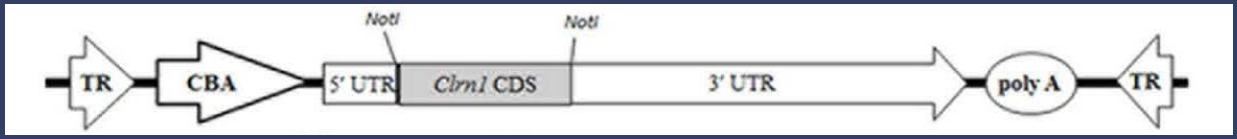
New model

-Delayed onset progressive HL (in CLRN1 null background)



GT Approach in Mice

Insert Clarin-1 cDNA into viral gene therapy vector



Inject gene therapy vector into new born mouse ear

Wait for a month

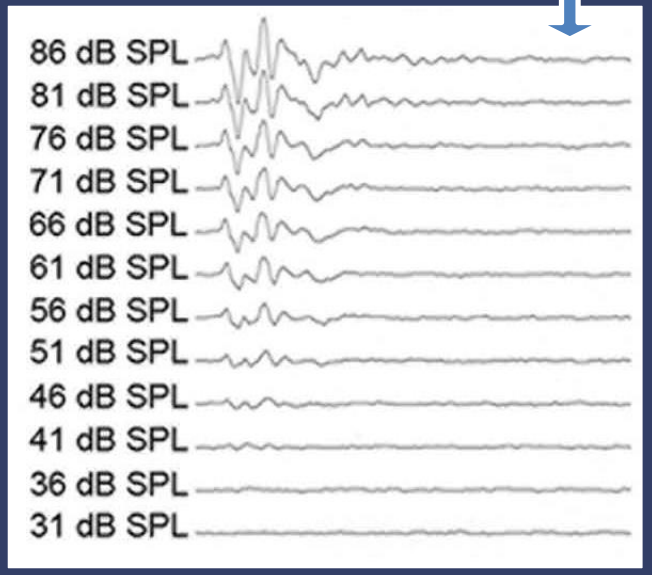
Start periodic hearing test from 1 month to 5 month
Compare hearing in treated vs. untreated siblings

Hearing test used: Auditory Brainstem Response

Sound → Ear → auditory nerve → brain(stem) →

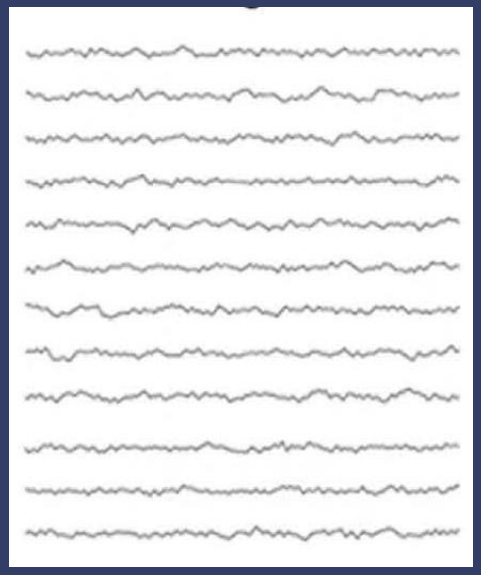
Waveform ← Recorder

Normal mouse



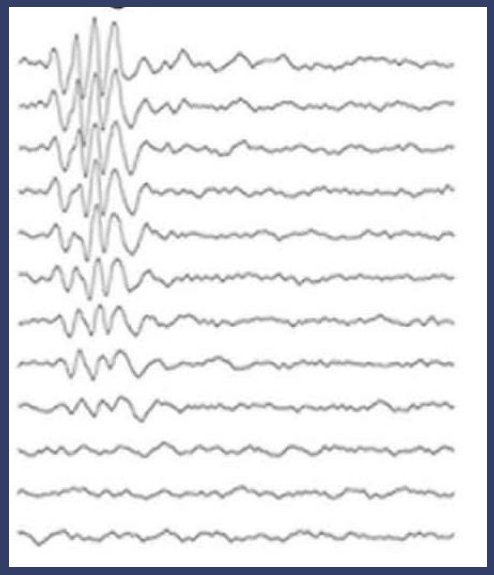
100 days old

USH3 mouse



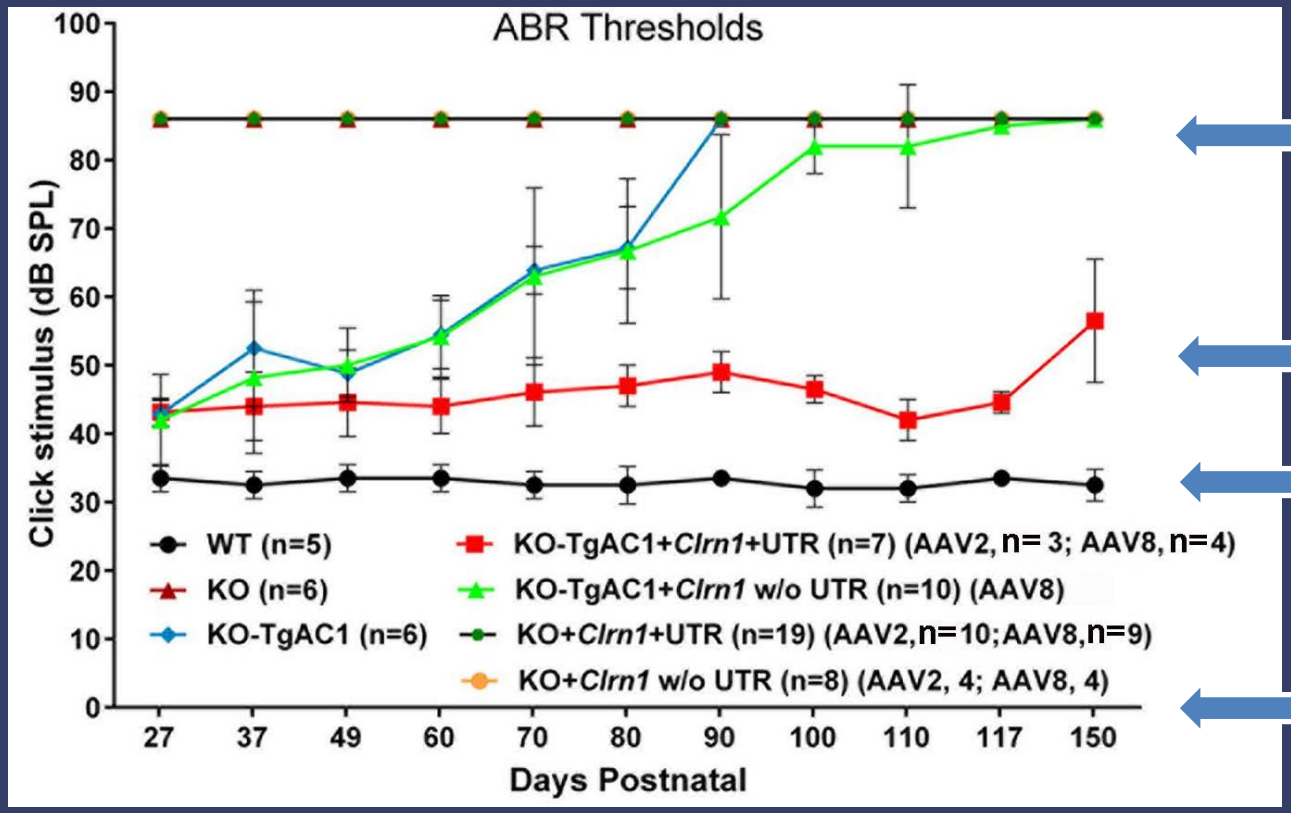
100 days old

GT USH3 mouse



100 days old

GT in USH3 mice is very effective!



(Untreated) USH3 mice

GT-USH3 mice

Normal mice hearing

Study stopped @ 150 days

Part II: Conclusions

- Developed a progressive HL mouse for USH3
- Developed a GT approach that is very effective in curtailing progressive HL in the USH3 mouse model and the effect remains stable
- GT vectors were introduced very early, i.e. before the onset of HL in this model

Next steps in GT for USH3

- Will GT work in the new USH3 mouse model if the viral vector was introduced in adult mice? If so, how well?
- Test GT in the USH3 model using new generation viral vectors, such as Anc80
- Apply for regulatory approval for a trial

General notes regarding my talk

- More than 10 years of research compressed into few slides.
- Both the small molecule therapy and gene therapy work represents team effort.
- Both publications are free online:
- https://www.ncbi.nlm.nih.gov/pubmed/29044151
- https://www.ncbi.nlm.nih.gov/pubmed/27110679

- Please contact me at kna3@case.edu if you have questions.

Acknowledgements

Funding

1. Usher III Initiative
2. NIDCD
3. University Hospitals Cleveland Medical Center


Cleveland Museum of Art
Ohio
Thank you