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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)

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)

Hair cells convert sound vibrations into electrical signals → to the brain via the auditory nerve.
Why HL with CLRN1 ∆?

What do the mouse models tell us?

Hearing in mouse models of UsH3

Hair cells - normal mouse

Hair cells - CLRN1 ∆ 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

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

2\textsuperscript{nd}, CLRN1^{N48K} is “stuck” inside the cell

Tian et al 2009; Geng et al., 2012
How to mitigate the CLRN1\textsuperscript{N48K} problem?

**Hypothesis**: Increasing the stability of CLRN1\textsuperscript{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\textsuperscript{N48K} stability in cell culture, but we cannot use these as drugs for disorders like USH3 because it is toxic.
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

![Diagram of small molecule structure](image)
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”

O03 stabilizes CLRN1^{N48K}, improving its chances of rendering clarin-1 mediated function in the cell
Optimized small molecule

O03 $\rightarrow$ Lot of work $\rightarrow$ BF844

$\sim2$ years

EC$_{50}$ 2 $\mu$M $\rightarrow$ EC$_{50}$ 0.34 $\mu$M
Testing the efficacy of BF844

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

We arrived at the doses based on \textit{in vitro} and \textit{in vivo} testing.
Hearing test used: Auditory Brainstem Response

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

Normal mouse

USH3 mouse

Treated USH3 mouse

Waveform

100 days old

100 days old

100 days old
BF844 mitigates HL in CLRN1^{N48K} Mice

Hearing improved in treated mice by 10,000 fold!
Small Molecule BF844

- 1\textsuperscript{st} targeted therapy for an Usher \(\Delta\)
- Preserves hearing in CLRN1\(^{N48K}\) mouse
- Since the CLRN1\(^{N48K}\) \(\Delta\) 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?

Preclinical studies

Clinical trials

CLRN1-N48K Subjects

~10 years Of Work!

Blocks PHL

Tg;KI/KI

BF844
Part II of my talk: Exciting Development!

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

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 $\Delta$ 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

USH3 mouse

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.
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