Therapies for Usher Syndrome

Update to Families

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#USH2018
Usher Syndrome

Cosgrove and Zallocchi, 2014, IJBCB
Gene

Protein

Cell

Organ
Therapies for Usher Syndrome

Gene augmentation therapy

Small molecules & pharmacology

Gene editing

Correction of translation
Gene augmentation therapy

**Alberto Auricchio**, Naples, IT
“Dual AAV vectors for gene therapy of USH1B retinitis pigmentosa”

Gene editing

**Carla Fuster García**, Valencia, ES (# 34)
“USH2A Gene Editing Using the CRISPR System”

Antisense and translational read-through therapy

**Erwin van Wijk**, Nijmegen, NL
“Antisense oligonucleotides for the treatment of Usher syndrome caused by splice site mutations”

**Jennifer J. Lentz**, New Orleans, US (# 37)
“Antisense Therapy Rescues Hearing and Vision in Usher syndrome”

**Kerstin Nagel-Wolfrum**, JGU Mainz, DE
“Translational read-through as therapy for Usher syndrome caused by nonsense mutations”
Small molecules and pharmacology

**Yoshikazu Imanishi**, Cleveland, US
“A small molecule mitigates hearing loss in a mouse model of Usher syndrome III”

**Alaa Koleila**, Rochester, MN, US (# 36)
“Development of the first pharmacotherapy for the treatment of Usher Type I due to variants in MYO7A”

Stem cells

**Mike Cheetham**, London, UK
“Retinal organoids as disease models”

**Anai Gonzalez Cordero**, London, UK (# 31)
“Using hiPSC-derived retinal organoids to model Ush2a pathophysiology”
**December 2017: First retinal gene therapy is approved**

*Dr. Jean Bennett*

On December 19, 2017, the U.S. Food and Drug Administration approved a new gene therapy (AAV2-hRPE65v2Luxturna), manufactured by Spark Therapeutics in Philadelphia.

Luxturna is the first gene therapy approved in the United States that’s directly administered into the eye, targeting diseases caused by mutations in the gene RPE65. Mutations in this gene can produce Leber’s congenital amaurosis or retinitis pigmentosa, both rare but potentially blinding diseases.
December 2017: First retinal gene therapy is approved

https://www.youtube.com/watch?v=jTVW-E5Cw2U
Gene augmentation
Adeno-associated virus (AAV) vectors for the expression of large transcripts
1- Dual AAV vectors transduce mouse and pig photoreceptors

Colella et al, Gene Therapy (2014) 21, 450–456
2- Dual AAV vectors restore melanosomes and rhodopsin localization in the retina of a mouse model of USH1B

Shaker mice (Myo7a mutant)
Dual AAV vectors restore morphological features in the retina of a mouse model of USH1B

- Treatment leads to correctly localized melanosomes

- Limits Rhodopsin accumulation at the connecting cilium

Trapani et al, EMBO Mol Med. 2014 Feb;6(2):194-211
Objective:
To test the safety and efficacy of a highly innovative gene therapy approach (dual AAV) in the retina of USHIB patients.

Coordinator:
Fondazione Telethon
Jennifer lentz (USH1C) 
"Antisense oligonucleotides effectively treat Usher Syndrome in mice."

Frame shift mutation found in French-Acadian USH1C patients of Louisiana. Results in a severely truncated protein and affects all harmonin isoforms.
Gene

Antisense Oligonucleotide

Protein

Cell

Organ

CRYPTIC SPLICING
Delivery of ASOs in Ush1c mice

Hearing and Balance
Target: Hair Cells

Systemic
- Intraperitoneal Injection (IP)
- Sub-cutaneous Injection (SC)

Local
- Tympanic membrane (TM)
- Topical-TM
- trans-TM
- RWM

Vision
Target: Photoreceptors

Local
Intravitreal Injection (IVI)
Systemic and local ASO treatment rescues balance behavior

Open-field Chamber

Systemic Treatment

Local Treatment

Lentz et al 2013
Correlation of translation

Erwin van Wijk, Nijmegen, NL

Antisense oligonucleotides

Exon skipping: **USH2A exon 13**

mRNA

- - exon 12 exon 13

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**USH2A mRNA**

c.2299delG (p.E767SfsX21)

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**AON**

- - exon 12 exon 13 exon 14

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**In frame skipping**

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Exon 12

Exon 13

Exon 14

Function

**++**

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**-**

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**+ ?**

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Exon 12

Exon 13

Exon 14

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Exon 12

Exon 14
Splice-modulation to treat USH2A-associated RP

QR-421a AON

Zebrafish *ush2a* exon13 knockout:
- Functional rescue!

&

Patient-derived photoreceptor progenitors
- Specific, non-toxic, effective

Save vision!

Preparation of phase1/2 clinical trials: anticipated to commence at end 2018

ProQR Therapeutics
Correction of translation

Kirsten Nagel Wolfrum
“Translation read-through to treat hereditary retinopathy”

TRIDS: Drugs that target in-frame non-sense mutations (premature stop)-
Most studied: Amynoglycosides

pR155X USH1C in-frame non-sense mutation (harmomin: red)

A

- wildtype
- PTC mutation
- read-through

B

- harm_a1-WT
- harm_a1-p.R155X + NB84
- harm_a1-p.R155X + PTC124
**USH2A**

**Correction of translation**

Kirsten Nagel Wolfrum  
*“Translation read-through to treat hereditary retinopathy”*

**USH2A:**  
W3955X most common in german population  
No expression of Usherin (USH2A protein)  
And G3142X mutation

See recovery of expression if treated with TRIDS (Ataluren) at a dose dependent manner. Importantly, we can see expression in patients derived fibroblasts after treatment with Ataluren.

Retinal pigment epithelium cells (RPE) from RP2 R120X patient derived fibroblasts
Correction of translation

A pharmacogenetic therapy for targeting nonsense mutations in USH - Ataluren

**Efficacy in transient transfected cells (USH2A, USH1C)**
- Restored protein expression
- Functionality

**Biocompatibility**
- Limited retinal toxicity (mouse; human)
- Phase 1 completed

**Efficacy in patient-derived cells**
- Protein expression
- Recovered localization
- Recovered ciliary phenotype
- Functionality

**Efficacy in animal models**
- Mice
- Pigs (USH1C_p.R31X)

**Clinical trial**
- Aniridia phase 2 (STAR; NCT02647359)
- FUTURE: Usher syndrome?
Retinal organoids as disease model

Mike Cheetham (UCL, London)

A. Efficient and Scalable production

Induced pluripotent stem cells (iPSCs) → Suspension culture → Seed embryoid bodies (adherent culture) → Cut out transparent neuroepithelium → Long term suspension culture

IPSC → embryoid body (suspension) → embryoid body (adherent) → neuroepithelium → retinal organoid

Parfitt, Lane, Ramsden et al Cell Stem Cell 2016
Burgoyne et al PLoS One 2018
Retinal organoids as disease model

Mike Cheetham (UCL, London)
PTC124 partially restores RP2 function in retinal organoids and is a good candidate for a clinical trial for RP2 stop mutations.

Retinal organoids as disease model

Parfitt, Lane, Ramsden et al Cell Stem Cell 2016
Dulla, Aguila et al Mol Therapy Nucleic Acids in press
Novel method for screening molecules

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

Improved molecule

New molecule

Proof of Concept!

Provided by Yoshikazu Imanishi
THE FUTURE IS IN OUR POWER!

SHINE A LIGHT ON USHER SYNDROME
a rare genetic disease causing combined deafness & blindness

Before their world is left dark and silent
The many faces of Usher Syndrome Research