Session 01 – Drug Delivery and Advanced Genetic Diagnostics

Diagnostic Evaluation for Hearing Loss in the Era of Gene Therapy

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BACKGROUND: A comprehensive diagnostic evaluation is required prior to administration of any gene therapy for patients with sensorineural hearing loss (SNHL). For pediatric patients with SNHL, imaging, congenital CMV testing, and genetic testing form the cornerstones of diagnosis. Although prevalence estimates for genetic SNHL approximate 50-60%, actual genetic testing yields average 43%. This discrepancy suggests a missing heritability in genetic SNHL using available sequencing methods. In addition, genetic testing may occur later than is optimal for treatment with gene therapy. Our lab is evaluating new methods to improve the diagnostic yield of genetic testing for hearing loss and to reduce time to diagnosis with genetic newborn hearing screening.
METHODS: We have recruited a cohort of pediatric patients with SNHL with varied audiologic phenotypes. We typically use a stepwise approach to diagnosis starting with either gene panels or exome sequencing. Short-read genome sequencing (srGS) and/or long-read genome sequencing (lrGS) are subsequently performed for undiagnosed patients. We have also begun a trial for newborns for whom we perform ultra-rapid comprehensive hearing loss genetic screening simultaneously with physiologic hearing screening.
RESULTS: 637 pediatric SNHL patients underwent genetic evaluation during the study period (2019-2024), including 234 gene panels, 465 exomes, 56 srGS, and 30 lrGS. The overall diagnostic rate was 30.9% with 42.3% for symmetric SNHL. Diagnoses were made in 65 genes, with *GJB2* (29.4%) and *STRC* (14.4%) being the most prevalent. 27.4% (n=54) of all diagnoses were syndromic. Gene panel diagnostic yield was 41.2% (n=63); performing ES on gene-panel negative probands added 9 additional diagnoses (13.0%). Diagnostic rate of srGS following ES was 1/56 (1.8%). LrGS following nondiagnostic srGS yielded 6/30 (20%). LrGS resolved variants in segmental duplications (*OTOA)* and genes with highly homologous pseudogenes (*STRC*). Results from a further ~50 patients sent for lrGS are pending. We have developed a platform for ultra-rapid genetic newborn hearing screening with automated analysis and results in less than 48 hours. We have performed this screening protocol on 105 subjects to date with enrollment ongoing.
CONCLUSIONS: When compared with gene panels, ES and srGS only marginally increase diagnostic yields. LrGS shows promise over srGS to better identify variants in difficult-to-sequence and noncoding genomic regions. Genetic newborn hearing screening holds promise as a method for earlier diagnosis for genetic hearing loss.

Microneedle-Mediated Safe and Precise Inner Ear Delivery

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Protected by one of the hardest bones in body, the cochlea is nearly an impenetrable structure frustrating both bacteria and clinician trying to gain access to it. As a result, a means for reliable delivery of agents into the inner ear for therapeutic purposes remains a formidable challenge. No method currently exists to provide effective and precisely dosed delivery of therapeutics to the inner

ear without risking permanent damage to the patient's hearing. We believe that an elegant solution to overcome the difficulties of intracochlear delivery is to use microneedles to facilitate reliable and predictable intracochlear delivery across the RWM without anatomic or functional damage. Intracochlear drug administration has been shown to be superior to transtympanic injection and results in significantly higher and less variable drug levels. In addition, there is a much smaller concentration gradient from base to apex, as is typical of transtympanic injection, resulting in a more even distribution of material. With the availability of a reliable method of inner ear delivery, targeted delivery to hair cells, spiral ganglion neurons or other intra-cochlear structures could be accomplished for the treatment of variety of auditory and vestibular disorders such as sudden or progressive SNHL, Ménière's Disease, and tinnitus.

Drug Delivery Studies in the Large Animal Model Pig

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Question: With its complex 3-dimensional structure, its seclusion deep within the temporal bone, and its isolation from the systemic circulation through the blood-labyrinth barrier, the cochlea remains a challenging target for drug delivery. Moreover, there is an evident lack of preclinical models, which can be used to study drug delivery techniques as well as vector delivery to the cochlea.

Methods: We established the pig as a promising large animal model to study cochlear drug delivery in a humanoid inner ear. Using this model, we investigated different delivery methods including intratympanic and intracochlear delivery, using different delivery appliances such as catheter systems, hydrogel or PLGA formulations, and explored the delivery of separate compounds and particles, such as steroids, viral vectors, or new molecules such as AC102.

Results: Initially, we studied the porcine cochlea's size using histological and micro-CT analysis and found its basilar membrane's length of roughly 33mm closely resembling the human's, while its volume exceeding rodent cochleae's volumes by up to 10-fold. Subsequently, we investigated and compared different delivery drug techniques to the pig's cochlea. We found that formulation of drugs such as dexamethasone into carriers, which provide sustained drug release, e.g. hydrogels or PLGA-implants, can effectively prolong residual time of the compound into the cochlea. Alternatively, changing the delivery technique itself can influence intracochlear drug distribution, such as the use of an intracochlear delivery catheter boosting apical and total cochlear drug levels. Furthermore, by studying the innovative new molecule, AC102, which possesses highly different molecular properties compared to conventional otoprotective drugs, we observed interesting distribution patterns within the cochlea and its sensory and neural tissues, which may revolutionize the way we study inner ear drug delivery. Lastly, the pig model was found to be suitable to study viral vector-mediated cochlear transgene delivery. Here, different spatial and cellular transduction patterns were found depending on the specific serotype of viral vector used.

Conclusion: The pig cochlea's anatomical and physiological similarity to the human inner ear renders this model as a highly suitable large animal model to study cochlear drug and viral vector delivery. With the use of this model, we are able to close the apparent translational gap between small animals and humans facilitating research and translation of new delivery strategies and gene therapy approaches.

Session 02 – Current Gene Therapy Trials

SENS-501 Gene Therapy in Young Children with Severe to Profound Hearing Loss Due to Otoferlin Mutations

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Background: Otoferlin mutation is responsible for 2-8 % of congenital severe to profound non syndromic hearing loss.
Objectives: This First in Man study intends to assess safety, tolerability, and efficacy of SENS-501 following unilateral intracochlear injection in children between the ages of 6-31 months with hearing loss due to a mutation in the Otoferlin gene.
Methods: The study consists of a dose-escalation part including two cohorts of three patients each, assessing sequentially a low and high dose of SENS-501 followed by a dose-expansion cohort at the selected dose recommended by the Data Monitoring Committee. While safety is the primary endpoint of the dose escalation study, Auditory Brainstem Response, twelve months following the injection, will be the primary endpoint for the dose expansion part
Results: To date 5 patients have received an intracochlear gene therapy injection (SENS-501). SENS-501 and the corresponding surgical procedure were uneventfully and well tolerated by all participants (aged 6 to 31 months and naive of cochlear implants at the time of the injection, as per study protocol) with no serious adverse events reported.
In Cohort 1 investigating the low dose, in one patient aged 11 months at the time of injection, early signs of hearing improvement were observed 3 months post injection using standard hearing tests. A positive response at ABR (Auditory Brainstem Response) was reported at two frequencies, with the best frequency reaching 70 dB compared to absence of response at baseline. An improvement of hearing levels of the Pure Tone Audiometry (PTA) across two frequencies with best frequency reaching 90 dB level compared to absence of response at baseline. Meaningful changes in responses to sounds and voices were reported by the parents with an IT-MAIS score increase of 16 points (145% relative improvement from baseline), and met expected auditory milestones based on an age-based parent questionnaire and according to the patient's age (LittlEARS).
Conclusions: The surgical procedure and perioperative period for each patient was uneventful. SENS-501 shows a good safety profile without any dose limiting event and preliminary positive data from the first cohort. The next step is to complete the recruitment of the second cohort at a higher dose.

Safety and Efficacy of DB-OTO Gene Therapy in Children with Profound Deafness due to Otoferlin Variants: Data from the CHORD Phase 1/2 Open-label Trial

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This talk will focus on DB-OTO. DB-OTO is a dual AAV1 vector designed for intracochlear delivery of *OTOF* to treat infants and children with profound deafness due to pathogenic *OTOF* variants. Twelve participants have been enrolled to date: 9 were dosed unilaterally and 3 were dosed bilaterally. DB-OTO was well tolerated, with no DB-OTO–related AEs or SAEs. The delivery has been well tolerated overall, with only transient vestibular AEs. 10 out of the 11 participants with ≥1 post-treatment assessment showed improved hearing. Among 5 participants with Week 24 assessments: 3 participants showed improvement in hearing thresholds to ≤40 dBHL and 2 participants showed improvement in hearing thresholds to ≤25 dBHL. In unilaterally treated participants, no ABR or PTA

response was observed in the contralateral untreated ear (with the cochlear implant turned off when applicable). In the first participant, there were clinically meaningful improvements in the DB-OTO–treated ear across tested frequencies. Hearing thresholds were within normal limits in most speech-relevant frequencies (0.5–2.0 kHz) and were associated with positive ABR responses. Formal speech perception testing without a device was measured at 22 and 27 months of age; preliminary results are encouraging and are corroborated by the family's observations. The CHORD trial is enrolling in the US, UK, Spain and Germany (clinicaltrials.gov: NCT05788536).

Clinical Development of AK-OTOF Gene Therapy for OTOF-mediated Hearing Loss: Preliminary Results

Aaron Tward
on behalf of the AK-OTOF trial and study site teams and Akouos, Inc. (a wholly owned subsidiary of Eli Lilly and Company)

Background: The otoferlin gene (*OTOF*) encodes otoferlin, a protein critical for signaling at inner hair cell synapses; individuals with *OTOF* mutations initially present with congenital, Severe to Profound sensorineural hearing loss, with preserved otoacoustic emissions. Advances in gene therapy and intracochlear delivery support potential hearing restoration in individuals with *OTOF*-mediated hearing loss using a one-time, local administration of AK-OTOF (AAVAnc80-hOTOF). This multicenter Phase 1/2 clinical trial (NCT05821959) evaluates the investigational medicinal product, AK-OTOF, and the investigational medical device, the Akouos Delivery Device, used to administer AK-OTOF to the intracochlear space.
Materials and Methods: Eligible participants have Profound hearing loss, as assessed by auditory brainstem response (ABR), at baseline and receive, using a minimally invasive transcanal approach, a single intracochlear administration of AK-OTOF in one ear. Safety assessments and hearing restoration, including by ABR and behavioral audiometry testing, are assessed over the one-year trial and an additional four-year long term follow-up period.
Results: The first participant, an 11-year-old, experienced restored hearing within 30 days of AK-OTOF administration; behavioral thresholds were 65 to 20 dB HL. The second participant, an 8-year-old, also experienced restored hearing within 30 days of AK-OTOF administration. The surgical administration and AK-OTOF were well tolerated, and no trial-related serious adverse events have been identified as of the date of this report. Safety and efficacy data from these and additional participants will be presented.
Conclusions: Interim data suggest that AK-OTOF may be safely administered to patients with onset of hearing restoration as early as one month following administration.

Gene Therapy vs Cochlear Implantation in Restoring Hearing Function and Speech Perception for Congenital Deafness Individuals

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Question: Gene therapy (GT), a novel treatment for congenital deafness, improves hearing and speech in patients with *OTOF* gene mutations. Among patients with congenital deafness, is GT more efficacious than CI in auditory and speech perception? To investigate the issue, we compared the efficacy of GT and CI for congenital deafness comprehensively.
Methods: This is a single-center, observational study. Participants, with congenital hearing loss, aged 1-18 years, received GT or CI, were enrolled. They were matched on duration of deafness, hearing threshold, and speech ability at the pre-surgical baseline. The primary outcomes include auditory and

speech perception evaluated by questionnaires including the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS)/MAIS, Categories of Auditory Performance (CAP), Speech Intelligibility Rating (SIR), and Speech, Spatial, and Other Qualities of Hearing Scale for Parents (SSQ-P) and tests including audiometry, speech perception in quiet or noisy environment, and music. The secondary outcome includes auditory information processing ability analyzed by mismatch negativity (MMN) recorded by electroencephalogram.

Results: Between December 2022 and November 2024, 72 participants were enrolled, including 11 GT patients and 61 CI patients. Participants received follow-up at 3, 6, or 12 months. Based on the hearing modality, participants were analyzed: GT-only patients vs matched CI patients, and bimodal patients (unilateral GT + contralateral CI) including GT+CI vs bilateral CI and GT (CI-off) vs unilateral CI. The average auditory brainstem response threshold was restored from >95 dB to 55 dB in 9 GT patients at 12 months. For GT-only vs CI, GT patients performed better in IT-MAIS/MAIS, SIR, SSQP-speech, SSQP-other qualities, and SSQP noise related scores at 6 months, and better in IT-MAIS/MAIS and CAP scores at 12 months. GT patients showed shorter latencies of MMN at 6 months. For bimodal patients at 12 months, GT (CI-off) patients performed better than unilateral CI patients in speech perception in noise; GT+CI patients performed better than bilateral CI patients in singing in-tune rates, and showed shorter latencies of MMN at 12 months.

Conclusions: GT patients show stable hearing recovery, more rapid improvement in auditory and speech perception, and better speech in noise as well as music perception, compared with CI patients. It is the first systematic study comparing the efficacy of GT and CI for congenital deafness, offering preliminary functional evidence for future clinical decision-making.

Session 03 – Stem Cell Therapies & Organoids

Mapping and Modeling the Human Inner Ear: RNA Atlases, Organoids, and Clinical Applications

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Our laboratory investigates the molecular mechanisms underlying human inner ear development and models this process using inner ear organoids derived from human induced pluripotent stem cells (iPSCs). This approach enables the study of early cell fate specification, the identification of key developmental pathways, refinement of organoid protocols, and the investigation of inner ear disorders and therapeutic strategies.
To provide a reference framework for normal development, we constructed HIEDRA, a single-cell RNA atlas of the human inner ear spanning the first and second trimesters. HIEDRA captures the emergence of sensory, supporting, neuronal, and secretory lineages, and will be made publicly available to support the broader inner ear research community. In addition to charting cellular diversity and developmental trajectories, the atlas highlights disease-relevant populations, offering insight into both congenital and acquired inner ear disorders.
Human inner ear organoids derived from iPSCs are emerging as a powerful in vitro platform to study sensorineural hearing loss. These 3D models recapitulate key aspects of human inner ear development and cellular complexity, potentially allowing investigation of many inner ear disorders. We applied this model to assess its potential for disease modeling and intervention. First, we demonstrated its use in studying ototoxicity caused by cisplatin and aminoglycosides. Second, we exposed organoids to congenital cytomegalovirus (CMV) infection to explore virus-specific inner ear tropism. Third, we modeled genetic forms of hearing loss using patient-derived iPSCs and tested therapeutic intervention through RNA-based antisense oligonucleotide (ASO) therapy.
Together, these efforts aim to establish robust, human-relevant inner ear models for understanding disease mechanisms and accelerating therapeutic development for auditory and vestibular disorders.

From Regenerating Auditory Neurons To Drug Screening

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Cochlear explants, the current gold standard for in vitro drug testing in the auditory field, are unsuitable for large-scale drug screens, as less than a dozen explants can be harvested from one animal. Billions of primary cell types would be needed to address all requirements needed to develop

a new drug. To introduce a new molecule as a clinical therapy in humans, several thousands of candidate molecules have to be screened in the first place, which is not realistic using the gold standard murine cochlear explant models.
We have previously identified and characterized the phoenix auditory neuroprogenitors (ANPGs) as highly proliferative progenitor cells isolated from the cochlea of a specific strain, called A/J mouse. These ANPGs have a virtually unlimited capacity to self-renew >40 generations. In a subsequent study, we aimed at identifying signaling pathways responsible for the intrinsic high stemness. A transcriptomic comparison of traditionally low stemness ANPGs, isolated from C57Bl/6 mice and high stemness phoenix ANPGs from A/J mice was performed. Based on the differentially expressed pathways, we reprogrammed low-stemness ANPGs with a strategic pharmacological combination of a WNT agonist and TGFβ/Smad inhibitors, which resulted in a remarkable increase in the growth of presenescent neurospheres, effectively allowing the expansion of ANPGs on an extensive scale. The so-called stemness-induced ANPGs exhibited the favorable property of being freezable and thawable, facilitating their distribution to other research facilities. Importantly, even after more than 20 generations, stemness- induced ANPGs retained their capacity to differentiate into electrophysiologically active type I-like auditory neurons.
Both the stemness-induced and phoenix ANPGs represent a significant breakthrough in addressing a major bottleneck in auditory research. They offer an efficient, high-throughput, cost-effective, and 3R compatible approach for in vitro screening of potential otoprotective and otoregenerative drug candidates. The next steps include to upgrade this platform incorporating human auditory neuroprogenitors and to transfer the methodology to hair cell, which remains a formidable challenge today.

Recent Advances in the Development of a Pluripotent Stem-Cell Based Therapy for the Treatment of Hearing Loss

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Disabling hearing loss is a growing global concern with significant social, personal, and economic impacts. While sensory hair cells are essential for hearing, mounting evidence suggests that the loss of neural connections between hair cells and the brainstem often precedes and exceeds hair cell damage, as seen in conditions like presbycusis. Auditory neuropathy is another example highlighting the importance of cochlear neural health, characterized by neuronal loss despite preserved hair cell function. Currently, no disease-modifying therapies exist; hearing aids and cochlear implants are the only available interventions. Cochlear implants can partially compensate for hair cell loss, but no regenerative treatment for cochlear neuron degeneration exists to date.
To address this, we are using human pluripotent stem cells to target the auditory nerve. We have shown that we can restore auditory thresholds in a gerbil model of auditory neuropathy by transplanting hESC-derived otic neuroprogenitors (hONPs) into the cochlear nerve. To model cochlear implantation, we used a fully implantable rodent stimulator with an electrode activated by a magnetic field. Such studies demonstrated functional integration between the transplanted cells and the implant.
To evaluate safety, we conducted long-term studies exploring the distribution and behaviour of transplanted cells. Animals were followed for up to a year, with whole-body MRI scans performed at termination to identify any potential lesions. Additionally, biodistribution studies for human-specific DNA sequences were performed by QPCR. No tumours attributed to the test article were found and there was no detection of cells spreading systemically. Similar safety studies were undertaken using the Rag2/Il2rg (SRG) double knock-out rat. Adapting the research-grade process to industrial manufacturing standards has not altered the properties of the cell product.

Rinri is now preparing to initiate a first-in-human trial using Rincell-1, a preparation of ONPs derived from human embryonic stem cells. A novel surgical approach for injecting Rincell-1 into the cochlear nerve via the round window has also been developed. The initial clinical trial will focus on assessing the safety and early efficacy of Rincell-1 as an adjunct to a cochlear implant in patients with neural hearing loss.