Special Issue on Hearing Therapeutics and Protective Therapies

Approaches to Treat Sensorineural Hearing Loss by Hair-Cell Regeneration: The Current State of Therapeutic Developments and Their Potential Impact on Audiological Clinical Practice

Ashley S. Hinton, AuD[1] Aizhen Yang-Hood, MD[2] Angela D. Schrader, MS[2] Christopher Loose, PhD[1] Kevin K. Ohlemiller, PhD[2] Will J. McLean, PhD[1,3]

[1] Frequency Therapeutics, Lexington, Massachusetts
[2] Department of Otolaryngology, Central Institute for the Deaf, Fay and Carl Simons Center for Hearing and Deafness, Washington University School of Medicine, Saint Louis, Missouri
[3] Department of Surgery, University of Connecticut School of Medicine, Farmington, Connecticut

Address for correspondence Will J. McLean, PhD, will.james.mclean@gmail.com

J Am Acad Audiol 2021;32:661–669.

Abstract

Sensorineural hearing loss (SNHL) is typically a permanent and often progressive condition that is commonly attributed to sensory cell loss. All vertebrates except mammals can regenerate lost sensory cells. Thus, SNHL is currently only treated with hearing aids or cochlear implants. There has been extensive research to understand how regeneration occurs in nonmammals, how hair-cells form during development, and what limits regeneration in maturing mammals. These studies motivated efforts to identify therapeutic interventions to regenerate hair-cells as a treatment for hearing loss, with a focus on targeting supporting cells to form new sensory hair-cells. The approaches include gene therapy and small molecule delivery to the inner ear. At the time of this publication, early-stage clinical trials have been conducted to test targets that have shown evidence of regenerating sensory hair-cells in preclinical models. As these potential treatments move closer to a clinical reality, it will be important to understand which therapeutic option is most appropriate for a given population. It is also important to consider which audiological tests should be administered to identify hearing improvement while considering the pharmacokinetics and mechanism of a given approach. Some impacts on audiological practice could include implementing less common audiological measures as standard procedure. As devices are not capable of repairing the damaged underlying biology, hair-cell regeneration treatments could allow patients to benefit more from their devices, move from a cochlear implant candidate to a hearing aid candidate, or move a subject to not needing an assistive device. Here, we describe the background, current state, and future implications of hair-cell regeneration research.

Keywords

- hearing loss
- regeneration
- therapeutic

received
March 13, 2021
accepted after revision
July 15, 2021

DOI https://doi.org/10.1055/s-0041-1750281.
ISSN 1050-0545.

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

Hearing Loss in Humans

In the United States, ~15% of adults 18 years and older report some difficulty in hearing (37.5 M).[1] In individuals aged 12 years or older, one in eight individuals in the United States (13%, or 30 million) has hearing loss in both ears based on standard audiological measures.[2] Worldwide, an estimated 1.1 billion individuals are at risk for disabling hearing loss.[3,4] Sensorineural hearing loss (SNHL) accounts for ~90% of all hearing loss cases.[5] SNHL has long been recognized as the primary and direct health effect of excessive noise exposure,[6] but can also arise from overexposure to ototoxic medications, viral/bacterial infections, acoustic neuromas, and even sudden unknown causes.

SNHL can impact people of all ages. Among adults aged 20 to 69 years, there is a substantial correlation of increasing age and hearing loss prevalence.[7] A recent study found that age-related hearing loss is primarily driven by hair-cell loss, and other damage observed in human temporal bone studies may not substantially contribute to the onset and manifestation of hearing loss.[8] Interestingly, this study also found that compared with aged-matched normal-aging ears, a lifetime of acoustic overexposure appears to be the primary cause for the observed damage.

SNHL has also been shown to correlate with impaired academic performance and social isolation at younger ages, and correlates with a higher incidence of dementia, depression, and other mental health disorders later in life.[9–12] Hearing aids and cochlear implants are the only rehabilitative options to address SNHL, but neither repairs the underlying biological deficit that cause the condition. Thus, a therapeutic that regenerates hair-cells to restore biological function within the cochlea has the potential to impact many patients and as a result, may alleviate the societal burden caused by untreated hearing loss.

Cochlear Function and Native Regenerative Capacity

Noise exposure, ototoxic drugs, and viral/bacterial infection are known to cause hair-cell damage and loss. It was long thought that hair-cell loss was permanent in vertebrates until studies in the 1980s discovered hair-cell regeneration occurred in birds after aminoglycoside and noise insult.[13–15] Around this time, it was also found that hair-cells regenerate in amphibians, reptiles, and fish. This suggested that the lack of hair-cell regeneration in vertebrates is strictly a mammalian limitation. Later work showed that some regenerative capacity exists in mammals during cochlear development and shortly after birth, but this ability is minimal and quickly lost, well before the onset of hearing.[16]

Hair cell damage is repaired in the ears of regenerative species through two processes: transdifferentiation and asymmetric division. Transdifferentiation occurs when supporting cells directly differentiate into hair-cells without first undergoing division.[15,17,18] Asymmetric division, however, gives rise to two daughter cells, with one acquiring a hair-cell fate. Preclinical studies have targeted both processes as potential methods to regenerate hair-cells.

While mammalian hair-cells lack any considerable regenerative potential, hair-cells and supporting cells share a common cellular precursor before their terminal commitment to either cell type. Thus, supporting cells are a primary target of hair-cell regeneration approaches. Research has gone into understanding the differences among supporting cells and their limited capacity to regenerate. During the organ of Corti's maturation, cell cycle inhibitor proteins of the Cip/Kip and Ink4 families are upregulated, which is shortly followed by hair-cell differentiation.[19] As these changes occur, the capacity for any passive hair-cell regeneration is lost.

Inducing Supporting Cells to Regenerate Hair-Cells in Preclinical Studies: Evidence to Move into Human Testing

Several different molecular targets have been reported to directly differentiate hair-cells, cause hair-cells and supporting cells to proliferate, or increase the regenerative capacity of supporting cells to divide and improve differentiation capability. This section of the review focuses on regenerative studies that evolved into clinical approaches to treat hearing loss.

Gene Therapy to Induce Atoh1 Expression to Regenerate Hair-Cells

Hair cell differentiation depends on upregulation of the gene *Atoh1*, a basic helix-loop-helix transcription factor. As a result, Atoh1 as a target to transdifferentiate supporting cells into hair-cells has been extensively studied. Work from 2005 described that viral transduction of supporting cells with Atoh1 adenovirus in guinea pigs in vivo produced new hair-cells after hair-cell ablation with an aminoglycoside.[20] This and a later study showed that the new hair-cells are immature or take on a "primordial fate" because they cannot be distinguished as cochlear or vestibular hair-cells.[21] Other studies showed that genetic upregulation of Atoh1 in a subset of supporting cells resulted in new hair-cell-like cells that fail to mature when performed in newborn mice, but when conducted in mature animals, any new hair-cell-like cells eventually die.[22] Later studies showed that Atoh1 induction did not result in improved auditory function in guinea pigs.[23] This suggests that Atoh1 upregulation on its own is not sufficient to restore proper functional hair-cells and indicates that other gene or molecular targets may be necessary to achieve this goal.

Inhibiting the Notch Signaling Pathway to Upregulate Atoh1 and Regenerate Hair-Cells

Atoh1 is in part regulated through the Notch signaling pathway, where active Notch signaling is known to suppress hair-cell formation. Notch signaling inhibition is known to drive hair-cell formation from supporting cells.[24–26] More recent efforts to restore hair-cells in vivo have focused on

upregulating Atoh1 with small molecules that inhibit Notch signaling to transdifferentiate supporting cells into hair-cells.[27,28] The use of small molecules has drug delivery advantages over gene therapy with viral vectors because small molecules can diffuse through the round and oval window membranes more readily due to lower molecular weight.[29] Mizutari et al (2013)[27] showed that intratympanic delivery in mice of LY411575, a γ-secretase inhibitor to inhibit the Notch signaling pathway and upregulate Atoh1, resulted in increased hair-cell numbers that appeared to be of supporting cell origin and improvements in auditory brain stem response (ABR) thresholds at low frequencies. Later reports suggested that Notch pathway components undergo downregulation prior to hearing onset in mice, which would reduce the ability of supporting cells to respond to γ-secretase inhibitors.[30] The Notch and Wnt pathways are two molecular signaling pathways that are well-established contributors to hair-cell development and regenerative capacity.[31] The Wnt pathway is involved in hair-cell development and differentiation,[32] and its upregulation can induce Atoh1 upregulation.[33] More recent work showed that induced Wnt pathway signaling upregulation can delay the downregulation of Notch receptors and their responsiveness to γ-secretase inhibitors, but these effects are limited to a few days.[34] This evidence suggests that Notch inhibition and Atoh1 upregulation alone may not be sufficient to regenerate hair-cells in mammals that have fully developed hearing.

Defining Distinct Cochlear Progenitors and Combination of Molecular Targets for Hair-Cell Regeneration

Recent work determined that distinct populations of progenitor cells exist within the postnatal mammalian inner ear that have defined capacities to form vestibular, cochlear, or neural cell types.[35] A subset of cochlear supporting cells expressing the surface receptor commonly referred to as Lgr5, a Wnt-associated epithelial stem cell marker,[36–38] serve as hair-cell progenitors during development.[35,39–41] Although Lgr5-expressing supporting cells cannot divide or regenerate after early embryonic stages,[42,43] later work showed that the combination of CHIR99021, a small molecule Wnt pathway activator, and the small molecule valproic acid (VPA) act synergistically to enable proliferation of quiescent cochlear hair-cell progenitor cells from mice, non-human primates, and humans.[40] In a mouse ex vivo ototoxic model, the combination of CHIR99021 and VPA (abbreviated as CV) induced supporting cells to divide and regenerate hair-cells.[40] These findings suggest that these progenitors can be induced to asymmetrically divide to replace themselves and form a new hair-cell. This is in contrast to the approach to directly convert supporting cells into hair-cells using Atoh1 gene therapy or Notch pathway inhibitors described previously, as this approach would eliminate these supporting cells.

To investigate the translational potential of this combination to improve hearing in the clinic, we applied CV intratympanically in an in vivo mouse model of noise-induced hearing loss (►**Fig. 1A**). Mice were exposed to extreme noise (120 dB SPL for 2 hours) similar to previously established protocols that induce near-immediate hair-cell death and hearing loss.[27,44] Drug was administered 24 hours after insult, similar to tests performed by Mizutari et al (2013)[27] to regenerate hair-cells with small molecules. When assessed 5 weeks after drug administration, ABR thresholds improved roughly 10 dB throughout the animals'

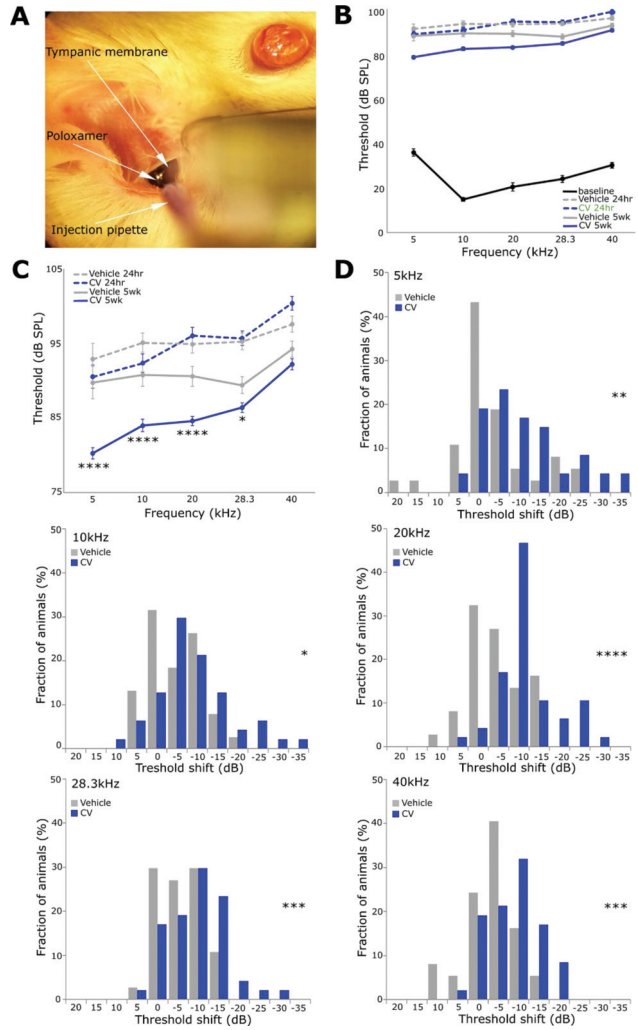

Fig. 1 Treatment with CHIR99021 + VPA (CV) leads to hearing improvement in an in vivo noise damage model. (A) Transtympanic injection of drug product into the middle ear of mice. (B) Animals designated to control-vehicle and CV groups had elevated thresholds at 24 hours and 5 weeks after noise exposure compared with pre-exposure baseline. Control $n = 37$ animals, treated $n = 47$ animals. (C) At 5 weeks after injection, CV animals had significantly lower hearing thresholds relative to control animals for four of the five frequencies tested. (D) The distribution of individual hearing recoveries was analyzed. Values represent the change in dB needed to elicit an auditory brain stem response (ABR), with positive values representing further threshold increases (further hearing loss) and negative values representing threshold decreases (improved hearing). The fraction of animals with a given ABR change from 24 hours to 5 weeks is shown for each frequency tested. The treated group had a higher incidence of animals with hearing improvement and the greatest individual recoveries. Values are presented as means ± standard error; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

hearing range (►**Fig. 1B**, **C**). The drug-treated group had more animals with larger threshold improvements across all frequencies than vehicle-treated (control) animals (►**Fig. 1D**). While not all animals responded to the drug, 77% of drug-treated animals showed a ≥10 dB threshold improvement compared with 30% of vehicle-treated animals. Further, some drug-treated animals improved their ABR thresholds by up to 30 to 35 dB (►**Fig. 1D**). These results correlated with a higher number of inner and outer hair-cell numbers (►**Fig. 2**). Although vehicle-treated animals showed a modest 4 dB threshold improvement, their hair-cell numbers after 5 weeks were similar to animals sacrificed 24 hours after damage, suggesting that this change could be due to temporary threshold shifts (not shown). Although the extent of recovery is likely limited due to extreme inner ear damage, these data gave evidence to support moving toward clinical development.

Status of Clinical Trials for Hair-Cell Regeneration

The data shown earlier provides evidence that hair-cell regeneration can occur using three different techniques. It also gives justification for moving into the clinic to test each approach's ability to improve hearing in human subjects. Below are updates on each method's clinical status at the time of drafting this review.

Novartis: Gene Therapy to Induce Atoh1 Expression to Regenerate Hair-Cells

Novartis initiated a Phase I/II trial to study the safety and tolerability of CGF166, their clinical recombinant adenovirus vector that contains cDNA of the human form of Atoh1 (Hath1). The adenovirus was delivered by intralabyrinth infusion under general anesthesia, which was administered by accessing the inner ear fluid by making a hole in the oval window footplate as the infusion site. The study enrolled subjects of both sexes between the ages of 18 and 75 years with unilateral or bilateral severe-to-profound hearing loss that was nonfluctuating, defined as having a pure-tone average within 10 dB of the pure-tone average obtained 11 months prior and a word recognition (WR) performance within 20% of the previous test obtained 11 months prior. Thresholds had to be ≤110 dB HL at 0.5, 1, 2, and 4 kHz, ≥50 dB HL for each testable octave frequency of 0.125 and 0.250 kHz, and ≥70 dB HL for each testable octave frequency from 0.5 through 8 kHz. Sentence recognition scores had to be ≤50% at screening.

The study enrolled 22 subjects and completed in late 2019. Subjects were not randomized and assigned to one of five treatment volume groups receiving CGF166. Primary outcomes were serious adverse events (AEs) and changes in pure-tone audiometry from 0.125 kHz through 16 kHz. Secondary outcome measures included changes in auditory evoked responses, assessments of vestibular function, and changes in speech recognition.

At the time of this article, there have been no formal updates on the progress or outcome of this trial. More study details and future updates can be found on the ClinicalTrials.gov Web site (https://clinicaltrials.gov/ct2/show/study/NCT02132130).

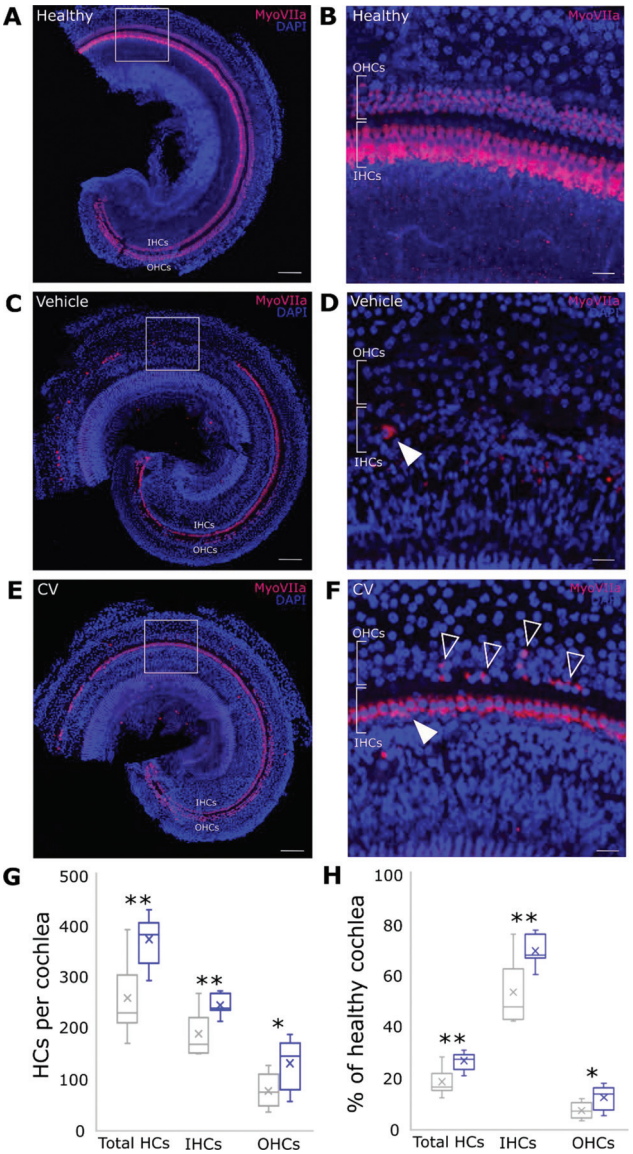

Fig. 2 Effects on hair cell number after treatment with CHIR99021 + VPA (CV). (A) Low magnification view of a healthy isolated cochlea showing complete rows of inner hair cells (IHCs) and outer hair cells (OHCs). (B) High magnification view of the region highlighted in (A) showing intact IHCs and OHCs in mid-frequency regions. (C) Cochleae of vehicle injected animals show widespread hair cell loss throughout the cochlea (apex and middle regions shown). (D) High magnification view of the region highlighted in (C) showing substantial absence of hair cells in mid-frequency regions, where a single IHC can be seen in the field of view (solid arrow). (E) Cochleae of CV-treated animals show a greater overall population of hair cells compared with vehicle-treated animals (apex and mid region shown). (F) High magnification view of the region highlighted in (E) showing a complete row of IHCs (solid arrow) and a population of OHCs (open arrow). (G) CV-treated cochleae (blue) show significantly more total hair cells, IHCs, and OHCs relative to vehicle-treated cochleae (gray). (H) The number of hair cells depicted as the percentage relative to an undamaged healthy cochlea. CV-treated cochleae (blue) show a significantly higher percentage of total hair cells, IHCs, and OHCs relative to vehicle-treated cochleae (gray). Scale bars, 100 μM low magnification, 20 μM high magnification. Values are presented as box-whisker plots; $n = 7$ animals per group; $^{*}p < 0.05$; $^{**}p < 0.01$.

Audion Therapeutics: Inhibiting the Notch Signaling Pathway to Upregulate Atoh1 to Regenerate Hair-Cells

In 2017, Audion Therapeutics initiated a Phase I/II multiple ascending dose open-label safety and efficacy study of the notch pathway inhibitor LY3056480, an Eli Lilly compound, in patients with mild to moderate SNHL in Europe. LY3056480 is a γ-secretase inhibitor that is designed to inhibit the Notch signaling pathway to upregulate Atoh1 to regenerate hair-cells, similar to the published preclinical study mentioned earlier using the Eli Lilly compound LY411575 in mice[27].

The study enrolled 59 subjects, male and female aged 18 to 80 years who had hearing loss of ≤20 years that suggested an age-related, noise-induced, or idiopathic origin. The subjects had to have symmetrical SNHL with <15 dB difference between ears and a pure-tone average across 0.5, 1, 2, 4, and 8 kHz between 25 and 60 dB HL, with two or more of those frequencies having thresholds less than 60 dB HL. Exclusion criteria included a primary complaint of tinnitus, presumed genetic or autoimmune causes of hearing loss, Meniere's disease, otitis media complications, or ototoxic therapies.

The main objectives were to assess the safety and tolerability of a local LY3056480 administration (intratympanic injection), and to study its efficacy at 6 and 12 weeks. Primary end points included local and systemic safety, as well as pure-tone changes from baseline and 12 weeks. Secondary end points included changes from baseline at 6 weeks, balance measures, tinnitus, facial nerve function and taste, and occurrence and severity of local and systemic AEs, such as electrocardiogram (ECG), vital signs, and laboratory tests. Specific details for trial sites in each country can be found at the given registry locations (https://www.clinicaltrialsregister.eu/ctr-search/trial/2016-004544-10/GB, https://www.clinicaltrialsregister.eu/ctr-search/trial/2016-004544-10/GR, and https://www.clinicaltrialsregister.eu/ctr-search/trial/2016-004544-10/DE). As of June 4, 2020, the REGAIN trial had reported that its primary end points were not met, but safety studies merited further evaluation of the product (https://cordis.europa.eu/project/id/634893/reporting).

Frequency Therapeutics: Combination Targets for Hair-Cell Regeneration

Frequency Therapeutics formulated CV into a clinical candidate named FX-322, which was shown to increase hair-cell numbers after damage ex vivo[40] and in noise-damaged adult mice (►**Figs. 1** and **2**). In a Phase 1b trial, 23 subjects were randomized to treatment with FX-322 via intratympanic injection ($n = 15$) or placebo ($n = 8$).[45] There were no notable differences in treatment-related AEs between the FX-322- and placebo-treated subjects. No drug-related systemic AEs occurred, and no clinically relevant changes were observed for clinical laboratory values, vital signs, ECG, otoscopy (with the exception of the single perforation), or tympanometry. No study participant, whether injected with FX-322 or placebo, showed a clinically meaningful decrement in hearing performance.

When examining data from the WR in quiet test, McLean et al (2021)[45] showed 13 of 23 participants scored 90% or better (excellent performance) at baseline and due to ceiling effects, could not be assessed for efficacy.[46] The 10 remaining participants (6 FX-322 and 4 placebo) scored less than 90%, allowing for efficacy assessment using Thornton and Raffin's binomial distribution. Four FX-322-treated ears showed statistically significant and clinically meaningful improvements from baseline to 90 days in the prespecified WR test,

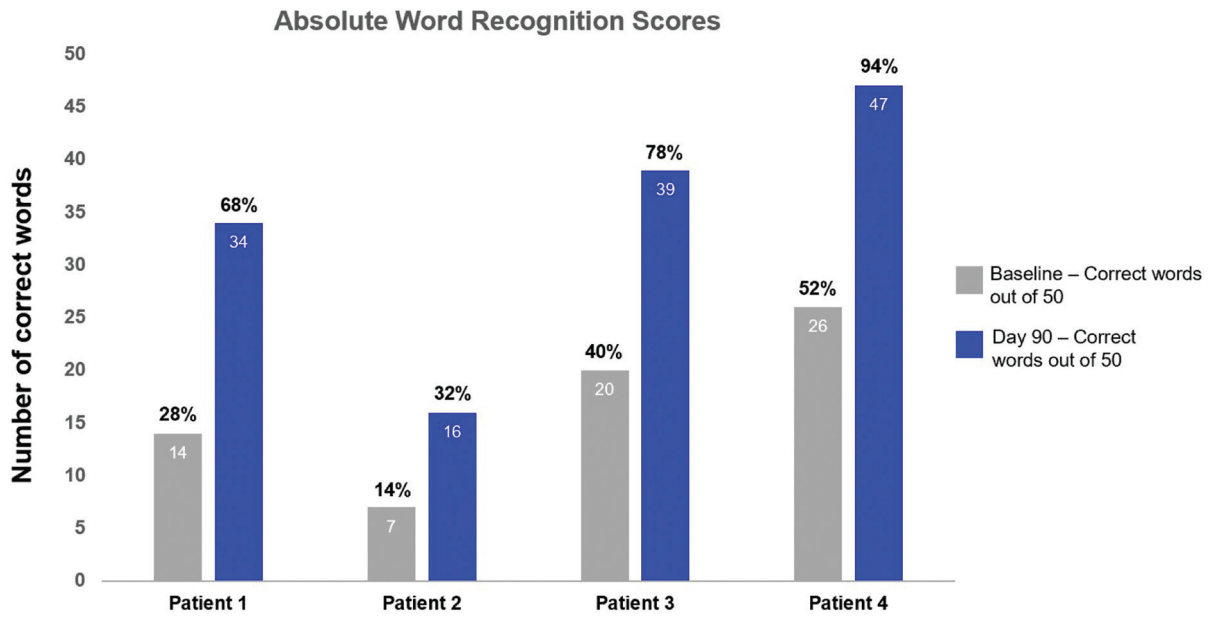

Fig. 3 FX-322 Phase 1b word recognition (WR) results. WR scores at baseline and day 90. Four FX-322 subjects and zero placebo subjects showed clinically meaningful and statistically significant improvement. (Adapted from McLean et al, 2021.[46])

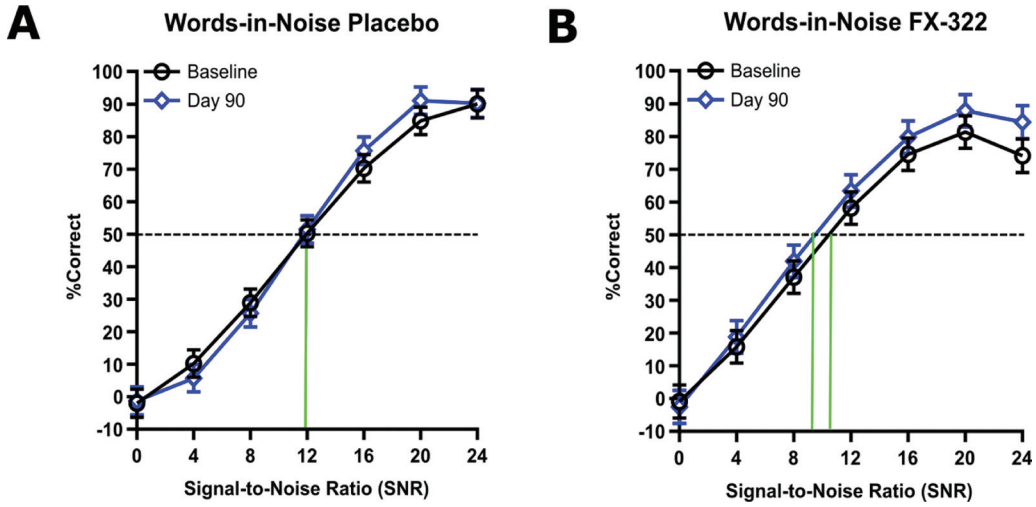

Fig. 4 Word-in-Noise (WIN) performance in subjects treated with an intratympanic injection of placebo or FX-322. (A) Psychometric functions for WIN show no improvement from baseline to day 90 for placebo-treated subjects ($n = 8$). (B) FX-322 ($n = 15$)-treated subjects show improvement (mean, 95% confidence interval, $p = 0.012$). (Adapted from McLean et al, 2021.[46])

exceeding expected levels of test–retest variability for this measure (►**Fig. 3**).[47] In contrast, no placebo-treated ears showed statistically significant changes (NCT03616223).

This clinical study also demonstrated that performance in the Word-in-Noise (WIN) test[48] also improved in some subjects treated with FX-322 but not placebo subjects. Performance was assessed using the signal-to-noise ratio (SNR; 0–24 dB) consistent with 50% correct, with lower SNR values indicating a better ability to perceive speech in background babble. Analyses showed a significant improvement in average SNR from baseline to day 90 in FX-322-treated subjects (−1.3 dB; $p = 0.012$) but not in placebo-treated subjects (−0.21 dB, $p = 0.71$) (►**Fig. 4A, B**).

The FX-322 clinical trial also reported subjects' scores across intelligibility measures. WR scores for the four subjects that exceeded the 95% confidence interval determined by Thornton and Raffin are shown along with their performance in the WIN test at baseline and the day 90 end point (►**Table 1**). These studies demonstrated that two of the four subjects had substantial and clinically meaningful improvements from baseline to day 90 in the WIN test, exceeding the 3.1 dB critical difference set forth by Wilson and McArdle.[49]

The four subjects mentioned earlier plus an additional subject that showed a trend in intelligibility improvement returned to the clinic for testing between 13 and 21 months after receiving FX-322. The mean (standard deviation) percentage of words correct in the treated ear was 38.4% (17.85) at baseline, 69.6% (23.04) at day 90, and 54.8% (21.05) at >1 year follow-up visit. Individually, three of five subjects maintained significant WR improvements compared with the original baseline as determined by the 95% confidence intervals for a 50-word test. These data, albeit of limited sample size, suggest that the improvement in WR observed is durable in some subjects with permanent SNHL for up to almost 2 years.

In addition to the functional intelligibility results obtained earlier, the work described in McLean et al (2021)[45] also examined the cochlear PK of FX-322 and its relation to pure tone measures based on preclinical perilymph measures, human perilymph measures, and validated PK modeling.[50] The PK results showed that the drug approaches therapeutic levels near 8 kHz but not lower frequencies. This is of importance because it could highlight the contributions of extended high frequencies (EHFs) in speech perception shown by others, particularly in patients with reported normal hearing but difficulty hearing in noisy environments coupled with EHF hearing deficits.[51,52] These results suggest that FX-322 could improve hearing performance, particularly speech perception.

Table 1 Intelligibility performance for most notable FX-322 responders

		WR		WIN		50% dB SNR	
		Baseline	Day 90	Baseline	Day 90	Baseline	Day 90
Subject	1	28%	68%	19%	33%	18.8	14.8
	2	14%	32%	26%	26%	18.8	18.8
	3	40%	78%	26%	40%	18.8	14.8
	4	52%	94%	47%	57%	12.8	10.0

Abbreviations: SNR, signal-to-noise ratio; WR, word recognition; WIN, words-in-noise in quiet.
Notes: Four patients showed notable improvements from baseline to day 90 in WR and WIN tests. Patients 1 and 3 showed clinically meaningful improvement in 50% dB SNR (a decrease ≥3.1 dB; 95% confidence interval).
Source: From McLean et al (2021).[46]

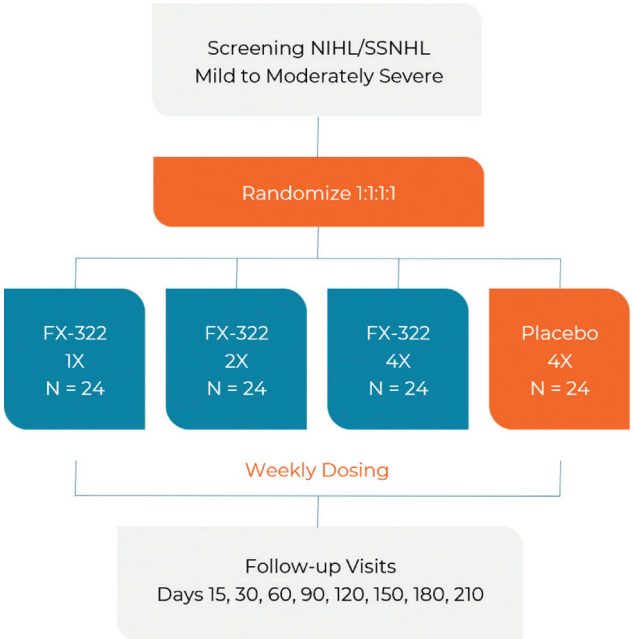

Fig. 5 **Phase 2a trial testing different dosing paradigms of FX-322.** All patients receive four injections weekly. Drug-treated subjects receive either one, two, or four doses of FX-322, with the remaining doses being placebo.

At the time of this writing, Frequency Therapeutics is conducting a Phase 2a trial to study FX-322 performance in defined hearing loss populations with different dosing paradigms. The study is outlined in ►**Fig. 5**.

Conclusion and Thoughts on the Future of Audiologic Testing

This publication outlines preclinical approaches that have advanced to clinical trials. The company, approaches, and proposed mechanism to generate new hair-cells are summarized in ►**Table 2**. As the biotech industry works toward interventions to treat hearing loss, many questions arise regarding assessing hearing function, classifying a hearing deficit, and indications for a clinically meaningful hearing improvement. At present, pure-tone audiometry is the gold standard for identifying and classifying hearing loss.[53] This is because devices target deficits in audibility to improve hearing performance, and audiometric testing provides a clinician with sound level targets for programming hearing devices. However, similar audiograms among patients can result in drastically different speech testing scores and varying satisfaction levels with assistive devices.[54] While speech recognition is part of comprehensive audiometric testing, it is often truncated (10- or 25-word lists rather than 50), or sometimes eliminated completely to save time in busy clinics.

However, results from Frequency Therapeutics' pharmacokinetic studies and clinical trials suggest that further tests of hearing function should be included to determine candidacy and assess efficacy for hearing regeneration therapies.[45] In a Frequency Therapeutics Phase 1b study in 2018, significant improvements were seen in speech recognition both in quiet and in noise.[45] In this trial, WR was performed using full 50-word Maryland CNC lists. Utilizing longer lists substantially reduces the variability in subjects' scores from visit to visit, thus allowing for better signal detection.[47] The WIN test[48] was used to assess speech-in-noise performance in this trial. Poor understanding in noise can contribute to the loss of sharp frequency-specific tuning provided by outer hair-cells.[51] Thus, restoring biological function to the inner ear could theoretically improve the auditory system's encoding of an amplified signal and necessitates speech-in-noise measures to assess the efficacy of intervention.

Pharmacokinetic data from Salt et al in guinea pigs show that drugs injected through the tympanic membrane enter the cochlea through the round window membrane and exit the cochlea with varying gradients, resulting in drug concentrations that are highest in the most basal region, or highest frequency regions of the cochlea.[55–57] This was recently investigated in humans where cochlear implant candidates received an injection of FX-322 prior to surgery and perilymph was collected through the round window membrane prior to electrode array insertion. Target concentrations of the components of FX-322 were confirmed in the basal region.[45] Recent work highlights the importance of auditory sensitivity in the EHFs while listening in background noise.[58–61] These results suggest that EHF audiometry may be of importance in a standard clinical test battery. Beyond behavioral measures of hearing function, physiological assessments such as otoacoustic emissions, ABRs, and electrocochleography provide objective measurements of hearing function and should be considered in a test battery to assess a patient for regenerative therapy. Existing approaches to collect these physiologic assessments would likely need to be modified from standard clinical practice to

Table 2 Summary of clinical trials utilizing hair cell regeneration approaches

Company	Therapeutic approach	Administration	Described mechanism
Novartis	Viral transduction	Intralabyrinth	Hath1 (Atoh1) gene therapy to directly convert supporting cells into hair cells
Audion	Small molecule compound	Intratympanic	Notch pathway inhibition to upregulate Atoh1 to directly convert supporting cells into hair cells
Frequency Therapeutics	Two small molecule compounds	Intratympanic	Wnt signaling pathway activator combined with sodium valproate to induce asymmetric division of supporting cells to generate new hair cells

get valuable information from diseased ears. Further research is needed in this area.

Drug development for hearing applications is in its infancy; hearing scientists, audiologists, and clinical specialists will need to work closely with experts from the biotechnology and pharmaceutical industries to design and execute robust clinical trials. In addition to considering how hearing professionals assess and classify hearing deficits to target potential subjects for treatment, we must also consider how we classify hearing improvement. There are several ways to analyze functional hearing data. While each method has value, each also has limitations that clinicians and scientists need to be cognizant of when interpreting audiologic data.

Percent change from baseline has long been an accepted metric for assessing drug efficacy in clinical trials. When considering a WR task, percent change from baseline is commonly assumed to be an absolute metric (i.e., 30–50% is a 20% absolute change). However, among drug development specialists, biostatisticians, and other experts in industry that are not as familiar with hearing assessment data, percent change from baseline is commonly assumed to be a relative metric that is dependent on baseline performance (i.e., 30–36% is a 20% relative change). Both methods are valuable and should be considered; conversely, they also have drawbacks. As hearing professionals, it is incumbent on us to be cognizant of this fact and educate our collaborators on the benefits and drawbacks of each method. For example, utilizing a relative percent change from baseline allows for normalization of baseline differences and efficacy assessment at the group level. However, the impact of relative percent change from baseline is distorted when baseline reference values vary widely, and is limited on the lower and upper ends of the distribution.

On the other hand, Thornton and Raffin's binomial distribution defines a statistically significant difference in an individual's WR score and has been the gold standard in hearing science for examining speech recognition performance. This method is also limited as the benchmark for statistically significant change is dependent on the variability of the distribution, which is small at the floor and ceiling and large in the middle. Further, the degree to which that statistical change correlates with a meaningful change in an individual's life has yet to be determined. The same issues arise when discussing meaningful improvements in pure-tone testing and speech-in-noise testing. There is generally no consensus in the hearing science community regarding clinically meaningful improvement in hearing function. This elucidates the need for further studies linking hearing assessment performance and quality of life (QOL) metrics. Validated patient-reported outcome (PRO) instruments would be of great benefit in determining the real-world impact of hearing therapeutics. At present, there are no Food and Drug Administration–approved QOL or PRO instruments for hearing.

As experts in hearing assessment and auditory interventions, evolving regenerative therapies give audiologists the opportunity to further establish themselves as vital practitioners for the evaluation, treatment, and management of candidates receiving biotherapeutic treatments for hearing loss. Currently, reimbursement for procedural codes encourages high throughput diagnostic testing and reflects the notion that hearing aids and cochlear implants are the primary interventions for SNHL. To move toward more thorough auditory assessment, the field will rely on audiologists to aid in the investigation, implementation, and if need be, the development of auditory measures to better assess this patient population, allowing for greater interdisciplinary collaboration among audiologists, biologists, auditory neuroscientists, and otolaryngologists.

Conflict of Interest
Ashley S. Hinton, Christopher Loose and Will J. McLean are all employees of Frequency Therapeutics.

References

1 Blackwell DL, Lucas JW, Clarke TC. Summary health statistics for U.S. adults: national health interview survey, 2012. Vital Heal Stat 10 2014;(260):1–161.
2 Lin FR, Niparko JK, Ferrucci L. Hearing loss prevalence in the United States. Arch Intern Med 2011;171(20):1851–1852
3 Cunningham LL, Tucci DL. Hearing loss in adults. N Engl J Med 2017;377(25):2465–2473
4 World Health Organization (WHO). Hearing loss due to recreational exposure to loud sounds: a review. 2015
5 Li L, Chao T, Brant J, O'Malley B Jr, Tsourkas A, Li D. Advances in nano-based inner ear delivery systems for the treatment of sensorineural hearing loss. Adv Drug Deliv Rev 2017;108:2–12
6 Basner M, Brink M, Bristow A, et al. ICBEN review of research on the biological effects of noise 2011-2014. Noise Health 2015;17(75):57–82
7 Goman AM, Reed NS, Lin FR. Addressing estimated hearing loss in adults in 2060. JAMA Otolaryngol Head Neck Surg 2017;143(07):733–734
8 Wu PZ, O'Malley JT, de Gruttola V, Liberman MC. Age-related hearing loss is dominated by damage to inner ear sensory cells, not the cellular battery that powers them. J Neurosci 2020;40(33):6357–6366
9 Choi JS, Betz J, Li L, et al. Association of using hearing aids or cochlear implants with changes in depressive symptoms in older adults. JAMA Otolaryngol Head Neck Surg 2016;142(07):652–657
10 Curhan SG, Willett WC, Grodstein F, Curhan GC. Longitudinal study of hearing loss and subjective cognitive function decline in men. Alzheimers Dement 2019;15(04):525–533
11 Deal JA, Betz J, Yaffe K, et al; Health ABC Study Group. Hearing impairment and incident dementia and cognitive decline in older adults: the Health ABC Study. J Gerontol A Biol Sci Med Sci 2017; 72(05):703–709
12 McGilton KS, Höbler F, Campos J, et al. Hearing and vision screening tools for long-term care residents with dementia: protocol for a scoping review. BMJ Open 2016;6(07):e011945
13 Cotanche DA. Regeneration of hair-cell stereociliary bundles in the chick cochlea following severe acoustic trauma. Hear Res 1987;30(2-3):181–195
14 Cruz RM, Lambert PR, Rubel EW. Light microscopic evidence of hair-cell regeneration after gentamicin toxicity in chick cochlea. Arch Otolaryngol Head Neck Surg 1987;113(10):1058–1062
15 Ryals BM, Rubel EW. Hair cell regeneration after acoustic trauma in adult Coturnix quail. Science 1988;240(4860):1774–1776
16 Cox BC, Chai R, Lenoir A, et al. Spontaneous hair-cell regeneration in the neonatal mouse cochlea in vivo. Development 2014;141 (04):816–829

17 Corwin JT, Cotanche DA. Regeneration of sensory hair-cells after acoustic trauma. Science 1988;240(4860):1772–1774
18 Roberson DW, Rubel EW. Cell division in the gerbil cochlea after acoustic trauma. Am J Otol 1994;15(01):28–34
19 Schimmang T, Pirvola U. Coupling the cell cycle to development and regeneration of the inner ear. Semin Cell Dev Biol 2013;24 (05):507–513
20 Izumikawa M, Minoda R, Kawamoto K, et al. Auditory hair-cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med 2005;11(03):271–276
21 Yang J, Bouvron S, Lv P, Chi F, Yamoah EN. Functional features of trans-differentiated hair-cells mediated by Atoh1 reveals a primordial mechanism. J Neurosci 2012;32(11):3712–3725
22 Liu Z, Dearman JA, Cox BC, et al. Age-dependent in vivo conversion of mouse cochlear pillar and Deiters' cells to immature hair-cells by Atoh1 ectopic expression. J Neurosci 2012;32(19):6600–6610
23 Atkinson PJ, Wise AK, Flynn BO, Nayagam BA, Richardson RT. Hair-Cell Regeneration after ATOH1 Gene Therapy in the Cochlea of Profoundly Deaf Adult Guinea Pigs. PLoS ONE 2014;09(07).
24 Bermingham NA, Hassan BA, Price SD, et al. Math1: an essential gene for the generation of inner ear hair-cells. Science 1999;284 (5421):1837–1841
25 Woods C, Montcouquiol M, Kelley MW. Math1 regulates development of the sensory epithelium in the mammalian cochlea. Nat Neurosci 2004;7(12):1310–1318
26 Zheng JL, Gao WQ. Overexpression of Math1 induces robust production of extra hair-cells in postnatal rat inner ears. Nat Neurosci 2000;3(06):580–586
27 Mizutari K, Fujioka M, Hosoya M, et al. Notch inhibition induces cochlear hair-cell regeneration and recovery of hearing after acoustic trauma. Neuron 2013a77(01):58–69
28 Tona Y, Hamaguchi K, Ishikawa M, et al. Therapeutic potential of a gamma-secretase inhibitor for hearing restoration in a guinea pig model with noise-induced hearing loss. BMC Neurosci 2014;15:66
29 Salt AN. Pharmacokinetics of drug entry into cochlear fluids. Volta Review 2005;105(03):277–298
30 Maass JC, Gu R, Basch ML, et al. Changes in the regulation of the Notch signaling pathway are temporally correlated with regenerative failure in the mouse cochlea. Front Cell Neurosci 2015;9:110
31 Waqas M, Zhang S, He Z, Tang M, Chai R. Role of Wnt and Notch signaling in regulating hair-cell regeneration in the cochlea. Front Med 2016;10(03):237–249
32 Shi F, Hu L, Jacques BE, Mulvaney JF, Dabdoub A, Edge AS. β-Catenin is required for hair-cell differentiation in the cochlea. J Neurosci 2014;34(19):6470–6479
33 Shi F, Cheng YF, Wang XL, Edge AS. Beta-catenin up-regulates Atoh1 expression in neural progenitor cells by interaction with an Atoh1 3′ enhancer. J Biol Chem 2010;285(01):392–400
34 Samarajeewa A, Lenz DR, Xie L, et al. Transcriptional response to Wnt activation regulates the regenerative capacity of the mammalian cochlea. Development 2018;145(23):145
35 McLean WJ, McLean DT, Eatock RA, Edge ASB. Distinct capacity for differentiation to inner ear cell types by progenitor cells of the cochlea and vestibular organs. Development 2016;143(23): 4381–4393
36 Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007;449 (7165):1003–1007
37 Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459(7244):262–265
38 Yin X, Farin HF, van Es JH, Clevers H, Langer R, Karp JM. Niche-independent high-purity cultures of Lgr5+ intestinal stem cells and their progeny. Nat Methods 2014;11(01):106–112
39 Chai R, Kuo B, Wang T, et al. Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. Proc Natl Acad Sci U S A 2012;109(21):8167–8172
40 McLean WJ, Yin X, Lu L, et al. Clonal expansion of Lgr5-positive cells from mammalian cochlea and high-purity generation of sensory hair-cells. Cell Rep 2017;18(08):1917–1929
41 Shi F, Kempfle JS, Edge ASB. Wnt-responsive Lgr5-expressing stem cells are hair-cell progenitors in the cochlea. J Neurosci 2012;32 (28):9639–9648
42 Lee Y-S, Liu F, Segil N. A morphogenetic wave of p27Kip1 transcription directs cell cycle exit during organ of Corti development. Development 2006;133(15):2817–2826
43 Ruben RJ. Development of the inner ear of the mouse: a radioautographic study of terminal mitoses. Acta Otolaryngol 1967;220:220, 1–44
44 Wang Y, Hirose K, Liberman MC. Dynamics of noise-induced cellular injury and repair in the mouse cochlea. J Assoc Res Otolaryngol 2002;3(03):248–268
45 McLean WJ, Hinton AS, Herby JTJ, et al. Improved speech intelligibility in subjects with stable sensorineural hearing loss following intratympanic dosing of FX-322 in a Phase 1b study. Otol Neurotol 2021;42(07):e849–e857
46 Lawson GD, Peterson ME. Speech Audiometry. San Diego, CA: Plural Publishing; 2011
47 Thornton AR, Raffin MJM. Speech-discrimination scores modeled as a binomial variable. J Speech Hear Res 1978;21(03):507–518
48 Wilson RH, Abrams HB, Pillion AL. A word-recognition task in multitalker babble using a descending presentation mode from 24 dB to 0 dB signal to babble. J Rehabil Res Dev 2003;40(04): 321–327
49 Wilson RH, McArdle R. Intra- and inter-session test, retest reliability of the Words-in-Noise (WIN) test. J Am Acad Audiol 2007; 18(10):813–825
50 Salt AN, Gill RM, Plontke SK. Dependence of hearing changes on the dose of intratympanically applied gentamicin: a meta-analysis using mathematical simulations of clinical drug delivery protocols. Laryngoscope 2008;118(10):1793–1800
51 Badri R, Siegel JH, Wright BA. Auditory filter shapes and high-frequency hearing in adults who have impaired speech in noise performance despite clinically normal audiograms. J Acoust Soc Am 2011;129(02):852–863
52 Motlagh Zadeh L, Silbert NH, Sternasty K, Swanepoel W, Hunter LL, Moore DR. Extended high-frequency hearing enhances speech perception in noise. Proc Natl Acad Sci U S A 2019;116 (47):23753–23759
53 Katz J. Handbook of Clinical Audiology. 7th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2014
54 Souza PE, Boike KT, Witherell K, Tremblay K. Prediction of speech recognition from audibility in older listeners with hearing loss: effects of age, amplification, and background noise. J Am Acad Audiol 2007;18(01):54–65
55 Plontke SK, Biegner T, Kammerer B, Delabar U, Salt AN. Dexamethasone concentration gradients along scala tympani after application to the round window membrane. Otol Neurotol 2008;29(03):401–406
56 Salt AN, Hartsock JJ, Hou J, Piu F. Comparison of the pharmacokinetic properties of triamcinolone and dexamethasone for local therapy of the inner ear. Front Cell Neurosci 2019;13:347
57 Salt AN, Plontke SK. Pharmacokinetic principles in the inner ear: Influence of drug properties on intratympanic applications. Hear Res 2018;368:28–40
58 Monson B, Buss E. Does extended high-frequency hearing matter in real-world listening? Hear J 2019;72:30–32
59 Monson BB. Benefits of extended high-frequency hearing for speech perception. J Acoust Soc Am 2020;148:2540–2540
60 Monson BB, Hunter EJ, Lotto AJ, Story BH. The perceptual significance of high-frequency energy in the human voice. Front Psychol 2014;5:587
61 Trine A, Monson BB. Extended High Frequencies Provide Both Spectral and Temporal Information to Improve Speech-in-Speech Recognition. Trends Hear 2020;24:2331216520980299

