ASGCT response to National Institute for Deafness and Communications Disorders RFI
Submitted March 31, 2020

1. What are the most significant scientific discoveries in hearing and balance, taste and smell, and voice, speech, and language that have occurred in the past five years? (Please provide references to scientific journal articles, if applicable.)

The American Society of Gene & Cell Therapy (ASGCT) is appreciative of this opportunity to provide input on NIDCD's strategic priorities. ASGCT is a nonprofit professional membership organization comprised of more than 3,500 scientists, physicians, and other professionals working in gene and cell therapy in settings such as universities, hospitals, and biotechnology companies. Our comments focus on the importance of research funding for gene therapy due to its considerable potential to treat hearing and balance disorders with significant unmet need. While genetic factors contribute to almost half of all cases of deafness, treatment options for genetic forms of deafness are limited. The genetics of congenital balance disorders are poorly understood, but multiple examples demonstrating dramatic recovery of balance phenotypes in mutant mice treated with gene therapy highlight the potential of this relatively unexplored space.

The most significant discoveries in the past five years include the successful application of gene therapy using adeno-associated viral (AAV) vectors to the inner ear. Zinn et al. first described the synthetic vector AAVAnc80 (2015, doi:10.1016/j.celrep.2015.07.019). In turn Landegger et al. (2017, doi:10.1038/nbt.3781) first reported using a serotype of AAVAnc80 to successfully transduce cochlear hair cells, followed by Nist-Lund et al. (2019, doi:10.1038/s41467-018-08264-w). Gyorgy et al. (2018, doi:10.1016/j.omtm.2018.11.003) reported successful cochlear transduction in mice utilizing an AAV9 capsid variant. These reports suggested that existing AAV-based gene therapy technologies could be adapted to the inner ear.

The development of strategies for restoring hearing and balance in mouse models has been reported in a number of recent studies. Askew et al. (2015, doi:10.1126/scitranslmed.aab1996) and Nist-Lund et al. (2019, doi:10.1038/s41467-018-08264-w) attained positive results via gene addition in mice with TMC-type mutations. Gene addition was also utilized with some success in mice with CLRN1 mutations, associated with Usher syndrome type 3A (Geng et al., 2017, doi:10.1038/s41598-017-13620-9). Across two academic groups, remarkable hearing rescue was achieved in in congenitally deaf, Otoferlin-deficient mice using a dual AAV approach to deliver this large gene (Akil et al., 2019, doi:10.1073/pnas.1817537116; Al-Moyed et al., 2019, doi:10.15252/emmm.201809396). These studies serve as proof of principle that the inherent inefficiencies of dual AAV approaches can be overcome in the closed compartment of the ear and are now being developed as therapeutics by several companies. In an alternate approach, Shibata et al. (2016, doi:10.1016/j.ajhg.2016.03.028) were able to prevent hearing loss in TMC1 mice via RNA interference. On the gene editing side, Gao et al. (2018, doi:10.1038/nature25164) and Gyorgy et al. (2019, doi:10.1038/s41591-019-0500-9) found CRISPR/Cas9 gene editing to

be effective on genetic hearing loss in mice with a dominantly inherited form of genetic deafness.

It was a breakthrough that Yoshimura et al. (2019; doi:10.1016/j.ymthe.2018.12.014) and Akil et al. (2019; doi:10.1073/pnas.1817537116) successfully applied gene therapy to adult mice. Previous studies were of gene therapies for neo-natal mice, whose hearing does not develop fully for several weeks after birth; since human hearing develops *in utero*, the ability to use gene therapy successfully in mature murine ears was a significant step toward its translation to human subjects.

Notably, the first clinical trial for hearing loss began in 2014, targeting the Atoh1 transcription factor. The trial, sponsored by Novartis and completed in December 2019, tested Atoh1 gene therapy for individuals with severe-to-profound unilateral or bilateral hearing loss to regenerate hair cells (ClinicalTrials.gov Identifier: NCT02132130). The trial's initial findings will assist the field in prioritizing endpoints and target populations for the next wave of regenerative therapeutics.

2. What are the unmet needs in current research and training that may impact hearing and balance, taste and smell, and voice, speech, and language?

Vector refinement and development for gene therapy are at the forefront of unmet needs in hearing research. Gene and cell therapies for hearing and balance disorders would particularly benefit from additional research into vectors with more efficient transduction capability, as well as evaluation of specific promoters for different cochlear cell types. It will also be important to determine which genes have multiple isoforms and what role they play; expanded knowledge of those isoforms specific to the inner ear is essential for effective gene therapy. Exploration outside the realm of conventional AAVs, which make up the majority of vectors used in hearing gene therapy research, may expand researchers' toolboxes to include synthetic AAVs, other types of viral vectors, or vectors that are non-viral in nature. Additionally, more research is needed on the development of base editors and guide RNAs (gRNAs) to correct missense mutations, as well as on development and targeting of prime editors and prime editing guide RNAs (pegRNAs) to correct insertion-deletion mutations. Research on utilizing gRNAs and pegRNAs in dividing cells would be especially beneficial.

The impact of preexisting immunity to common vectors in the inner ear is a key unknown for hearing gene therapies; insufficient data exist to determine how preexisting immunity may interfere with cochlear gene therapy administration. Whereas the disqualifying immune response threshold for systemic intravenous administration is relatively well understood, further research is required to determine levels for the unique environment of the inner ear.

Lack of relevant cell lines and *in vitro* cell models is another significant unmet need in current hearing and balance research. In addition, gene therapy research is impossible without effective preclinical models. ASGCT members believe it is critically important that improved, well-characterized animal models be developed for hearing and balance disorders, including common hereditary hearing disorders. Needs include the development of improved mouse models to

support preclinical proof-of-concept studies, as well as non-rodent models for translational and bridging studies. Very few non-rodent models and/or human tissue proxies can be used to bring products from animal models to humans in an effective manner. Although there has been striking success in transducing the cochlea in a nonhuman primate (Ivanchenko et al., 2020, doi:10.1016/j.heares.2020.107930), many more vectors need to be tested and toxicity evaluated.

Gene therapies for sensorineural forms of hearing loss would also benefit from more thorough natural history studies of human hereditary deafness and balance disorders. Such research would help determine the most effective treatment window for therapeutic intervention. Natural history studies could also illuminate the cochlea's transcriptome, enabling researchers to correlate genotype with phenotype in specific hearing disorders, identify treatable patient populations, and rationally design clinical trials. Particularly lacking is an understanding of the progression of histopathology in humans, which will be essential to identifying diseases with a viable treatment window. Since many mutations likely result in rapid deterioration of the ear prior to birth in humans, a concerted effort to collect more human temporal bone data will be key to advancing new therapeutics for patients. As patient identification and natural history understanding improves, a number of other issues may need to be addressed, including appropriate endpoints to demonstrate clinically meaningful benefit for various disorders and age groups.

3. Describe the opportunities in hearing and balance, taste and smell, and voice, speech, and language that may be realized in the next five years.

Gene therapies, including gene editing, have the potential to address a wide variety of genetic hearing and balance disorders. Like the eye, which was the target of the first *in vivo* gene therapy to receive FDA approval in the United States, the ear presents a small, enclosed environment that exists in relative isolation from other body systems. For this reason, high concentrations of a therapeutic can be delivered to target cells with minimal systemic exposure, offering an ideal setting for development of gene therapies.

Current genetic testing research has made clear the large number of genes that relate to hearing loss, which is both an opportunity and a challenge. Research on gene therapy for hearing loss has the potential to grow significantly with the development of effective tools and innovative therapies, and researchers have made great progress toward proof-of-concept studies. The safety and efficacy findings from ongoing and upcoming clinical trials of gene therapy for the ear may assist in the identification of additional potential genetic hearing and balance disorders for which gene therapy may be beneficial.

Techniques as varied as stem cell grafting, induced cell differentiation, and other approaches to hair-cell repair are in early exploratory phases. The development of mutation-agnostic gene therapy approaches would be a monumental advance. Other early studies have explored stimulating and regenerating synapses in cases of hidden hearing loss, in which an individual can pass a standard hearing test but cannot differentiate sounds in a noisy environment. As varied therapeutic avenues are discovered, entirely novel techniques may also be developed.

At a physiological level, much is still unknown about the functionality and development of the inner ear, particularly in individuals with congenital hearing loss. It is critical that researchers

have access to high-quality otopathologic data, like that stored at the three national otopathology laboratories. Patient registries would also be an effective way to illuminate population size within each diagnosis.

4. What are the greatest challenges or barriers to progress in hearing and balance, taste and smell, and voice, speech, and language?

While most genes associated with hearing loss are small enough to be packaged in a single AAV, there are a number that are too large. Studies in the last year have demonstrated some success with cochlear dual-vector delivery in mouse models (Akil et al., 2019, doi:10.1073/pnas.1817537116; Al-Moyed et al., 2019, doi:10.15252/emmm.201809396). Other techniques could include a gene-editing approach, which has shown early results (Gao et al., 2018, doi:10.1038/nature25164; Gyorgy et al., 2019, doi:10.1038/s41591-019-0500-9), or miniaturizing the gene. The challenge of packaging capacity will need to be overcome across gene therapy disciplines. Additionally, some gene therapies will require development of post-administration safeguards, in which a genetic off switch is activated to prevent indiscriminate gene expression. Therapeutic transgenes expressed in non-target cells could lead to safety concerns, and this will need to be better understood as hearing gene therapies are developed.

The isolation that makes the ear attractive for gene therapy also makes it difficult to access. This therapeutic accessibility "blind spot" impacts the ability to track therapeutic response and match the right patient with the right therapeutic approach, and impedes clinical trial design for many ear diseases. Imaging might involve a surgical probe approach, electrophysiological screening, or other techniques, but the sheer visual impassability of the inner ear has been a challenge for developing pharmacologic therapies, including gene therapies. The development of less invasive delivery of gene therapies to the inner ear could also be beneficial, either through simplified surgical approaches or through delivery systems that do not require injection directly into the cochlear fluid space.

As in all fields, nonhuman models and human tissue proxies are imperfect corollaries for human biology. Mice, for example, develop hearing in a similar manner but on an entirely different time course than humans, so may have limited utility beyond the proof-of-concept stage if an investigational therapy is administered before the postnatal maturation of the cochlea (which is prenatal in humans and nonhuman primates). Cultured human inner ear hair cells are an effective study subject when available, but they are difficult to culture and are extremely short-lived. Cochlear and inner ear organoids, which have come far in the last half decade (Roccio & Edge, 2019, doi:10.1242/dev.177188), are useful for drug screening but have innate limitations for more advanced research. Developing additional, and in some cases more accurate, mouse models in terms of reflecting the phenotype in humans, could be necessary to support further research. The availability of larger animal models of genetic hearing loss could also help facilitate translational activities.

ASGCT appreciates your consideration of these comments. Please do not hesitate to contact us if you have questions.