GJB2-GT, a Novel AAV-Based Gene Therapy as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 1A (DFNB1A)

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Abstract

Background: In the world, the estimated prevalence of severe or profound deafness in human is 1 out of 1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding for Connexin 26 (Cx26), are involved in 50% of congenital deafness and are mostly associated with an autosomal recessive non-syndromic DFNB1A. In the cochlea, GJB2 is largely expressed in the supporting cells (SCs) of the sensory epithelium, fibrocytes, and basal and intermediate cells of stria vascularis but not in sensory hair cells. It is hypothesized that Cx26 is essential for the recycling of potassium, which is essential for the proper functioning of sensory hair cells, but in vivo studies also suggest that Cx26 deficiency leads to cochlear developmental disorders. Gene therapy is a promising therapeutic strategy for autosomal recessive forms of deafness, and Adeno-Associated Vectors (AAVs) are being developed to this aim.

Methods: Here, we have developed GJB2-GT, an AAV vector for DNFB1A that offers broad coverage of Gjb2-expressing cells of the inner ear in both mouse and non-human primates and designed its specific expression cassette to allow detargeting of hair cells. GJB2-GT was delivered into congenitally deaf conditional Gjb2 mutant mouse ears through the round window (RW). Efficacy on on-going cohorts, dose-response experiments, correlation between ABR responses and transduction efficacy, early biodistribution and toxicology studies in mice were investigated. In parallel, GJB2-GT was administered to Non-Human Primates (NHP) using the surgical approach and medical injection device used in human. Early tolerability and biodistribution of GJB2-GT were assessed. Whole mounts and cryosections of injected inner ears were analyzed to assess the AAV tropism by immunoconfocal analyses and in situ hybridization.

Results: A single intracochlear injections of GJB2-GT into conditional Gjb2-mutant mouse inner ears lead to statistically significant improvement of hearing thresholds as early as 3 weeks post-injection in a dose dependent manner. In NHP, three weeks post-surgery, biodistribution was mostly limited to injected ears. In WT mice, GJB2-GT was also well tolerated. Lack of impact on ABR was demonstrated 6 months post administration together with long-term transgene expression. In mice and NHP, broad target cells coverage was observed, the vast majority of SCs were transduced along the tonotopic axis and no transduction was found in hair cells.

1. GJB2-GT allows for active Cx26 production with broad coverage and specificity for target cells

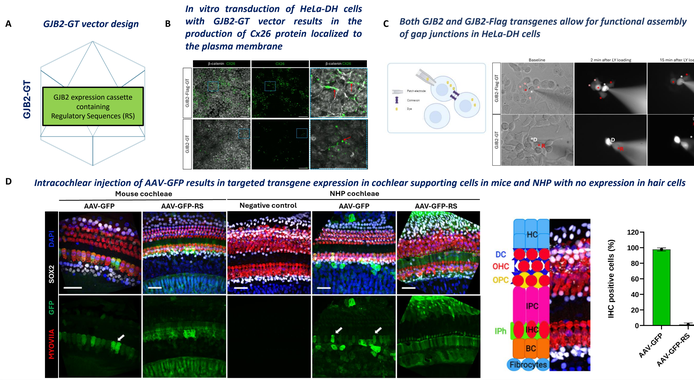


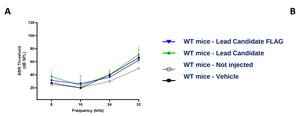
Figure 1: A. GJB2 vector design with regulatory sequences allowing de-targeting of hair cells. **B.** Hela DH cells transduced with GJB2-GT or GJB2-Flag-GT (MOI 1E+05) were immuno-stained with antibodies against CX26 (green) and β-Catenin (grey). Scale bar 50µm. Red arrows indicate **CX26 plaques at the intercellular interface, consistent with the formation of gap junctions C.** Dye transfer assay: gap-junction permeable fluorescent dye Lucifer Yellow (LY) is micro-injected into transduced HeLa-DH cells using a patch electrode. **LY spread to adjacent cells when injected in HeLa-DH transduced with GJB2-GT (lower panel) or GJB2-Flag-GT (upper panel), indicating the assembly of functional gap junctions**. **D.** Whole mount imaging of Organ of Corti from mice and NHP injected with AAV-GFP or AAV-GFP-RS. Arrows indicate transduced inner hair cells (IHC) Scale bar 40 µm. **GJB2-GT demonstrates broad coverage of target cells and no expression in IHC in mice and NHP.**

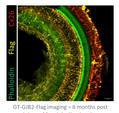
2. Summary of exploratory safety and biodistribution studies of GJB2-GT in mice and NHP

Acute toxicity in WT Mice - High dose IV injection

- Study performed in preparation of upcoming GLP-toxicity in mice after IV injection
- GT-GJB2 does not interfere with normal growth and don't elicit elevated transaminase levels 4 and 8 weeks after injection
- Behavioral evaluation - Functional Observation Battery, exploratory behavior (videotracking), 3 and 7 weeks after injection: no findings

6-Month Exploratory Safety and Transgene Expression in WT Mice – Intracochlear injection





- No impact on ABR up to 6 months
- Normal histology
- Transgene expression persistence
- Hair cells detargeted
- Clinical pathology: no findings

3-Month Exploratory Toxicity and Biodistribution in NHP - Intracochlear injection

- GJB2-GT is well tolerated and did not induce any macroscopic/organ weight changes
- No lab and clinical findings
- Biodistribution: the vast majority of the vector remains in injected ears, no dissemination observed in gonads, main organs, dorsal root ganglion (DRG)

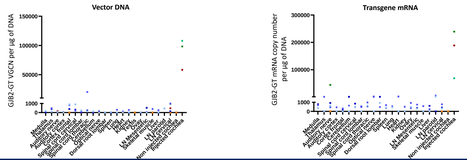


Figure 2: A. WT mice were injected with the indicated vectors through the RWM. Auditory brainstem response (ABR) were measured up to 6 month after vector administration. **GJB2-GT injected animals display normal ABR thresholds, consistent with good local tolerability of the vector B**. Whole-mount imaging of organ of Corti from WT mice injected with a flagged version of the GJB2-GT vector. The flag epitope is broadly detected in supporting cells, **indicating appropriate cellular tropism and maintenance of transgene expression up to 6 months post-injection. C.** Biodistribution of vector genome (left panel) and transgene mRNA (right panel) in the cochlea and peripheral tissues of NHP administered with GJB2-GT. Biodistribution studies were conducted 3 weeks, 1 month and 3 month after vector administration, as indicated. The sensitivity of the method allows for accurate quantification of at least 50 copies of vector genomes (VGCN, vector genome copy number) per microgram of genomic DNA, and 10 copies of mRNA in 10 ng of total RNA.

3. GJB2-GT administration in NHP as intended in human

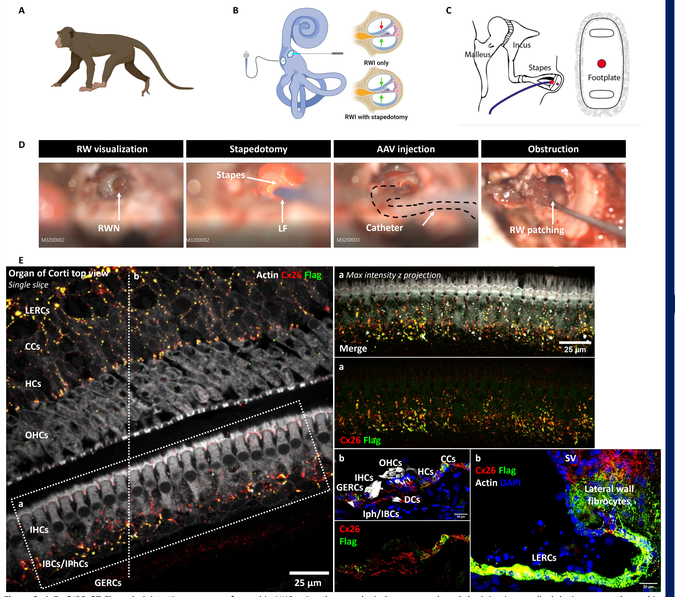


Figure 3: A-D. GJB2-GT-Flag administrations were performed in NHP using the round window approach and the injection medical device currently used in Sensorion's gene therapy clinical trials. Briefly, a transcanal approach is performed. After visualization of the footplate of the stapes, a stapedotomy is performed and an in-house designed catheter connected to a dedicated injection medical device is inserted in the round window membrane and the intracochlear administration performed. **E.** Three weeks post-injection, NHP were euthanized, the temporal bone collected and fixed in PFA. After decalcification, the cochleae were fine-dissected and immunostained using antibodies directed against Cx26 and the Flag tag epitope. Phalloidin counterstaining is used to detect actin. Max intensity projection of whole mount organ of Corti (a) as well as cochlea cryosections (b) are shown. **Using the surgery route and injection device envisioned for human, GJB2-GT Flag vector administration result in correct expression of Cx26 at the cell membrane of target cells with no expression in hair cells.**

4. Natural history of a new conditional mouse model for DFNB1A : Gjb2$^{cKO1/cKO1}$

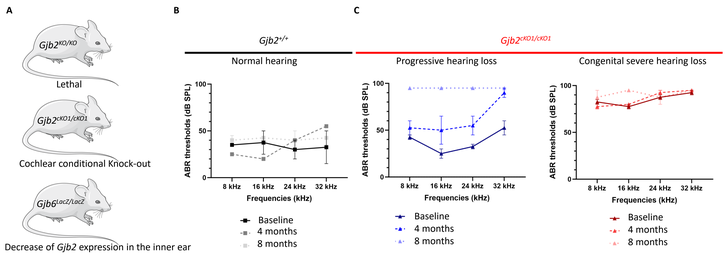
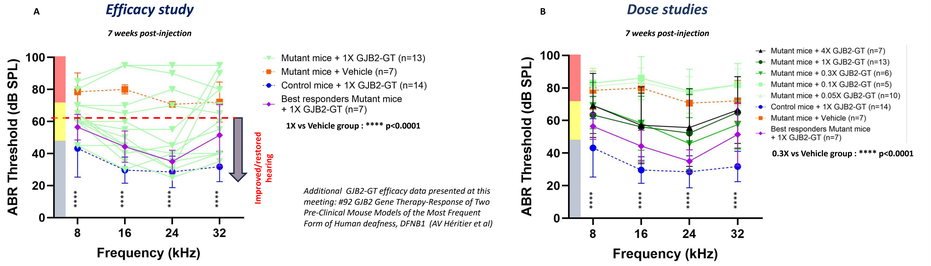


Figure 4: A. *Gjb2*$^{cKO1/cKO1}$ mutant mouse model is a useful tool to study DFNB1A physiopathology and to perform proof of concept (POC) studies since the homozygosity for *Gjb2*$^{KO/KO}$ allele is lethal during the embryonic development of the mouse. **B,C.** Assessment of the hearing function of *Gjb2*$^{cKO1/cKO1}$ mutant mice up to 8 months. Control mice from this strain show normal hearing and do not present age related hearing loss due to the absence of Cdh23AHL allele in the genetic background. *Gjb2*$^{cKO1/cKO1}$ mutant strain presents two different hearing loss phenotypes: 1/ Progressive mutant mice show hearing loss from 4 months to reach severe/loss hearing loss and 2/Congenital severe hearing loss animals present ABR above 80dB thresholds. **Our ongoing efficacy studies currently focus on the congenital form**

5. Ongoing efficacy and dose studies using RWI of GJB2-GT in Gjb2$^{cKO1/cKO1}$ mutant mice



Additional GJB2-GT efficacy data presented at this meeting: #92 GJB2 Gene Therapy-Response of Two Pre-Clinical Mouse Models of the Most Frequent Form of Human deafness, DFNB1 (AV Héritier et al)

Figure 5: A. Efficacy study. ABR recordings for GJB2-GT injected (individual – green) or vehicle (average – orange) *Gjb2*$^{cKO1/cKO1}$ mutant mice and for GJB2-GT injected *Gjb2*$^{+/+}$ control mice (average – blue 7 weeks after intracochlear injection. Purple traces represent average ABR measurements for the 7 best responder animals, presenting average ABR thresholds across tested frequencies ≤ 60 dB (red dotted line.) **GJB2-GT induced a statistically significant hearing recovery for at least 7 weeks after injection in *Gjb2*$^{cKO1/cKO1}$ mutant mice.** Colored zones represent approximative profound (>70 dB, red), moderate (45-70 dB, yellow) and no (<45 dB) hearing loss thresholds. **B, Dose studies.** Average ABR recordings for GJB2-GT 4X to 0.05X injected (black to light green) or vehicle (orange) *Gjb2*$^{cKO1/cKO1}$ mutant mice and for GJB2-GT injected *Gjb2*$^{+/+}$ control mice (blue) 7 weeks (D) after injection. Purple traces represent average ABR measurements for the 7 best responder animals, presenting average ABR thresholds across tested frequencies ≤ 60 dB at dose 1X. **A 0.3X dose of GJB2-GT is sufficient to induce a statistically significant hearing recovery for at least 7 weeks after injection in *Gjb2*$^{cKO1/cKO1}$ mutant mice.** Colored zones represent approximative profound (>70 dB, red), moderate (45-70 dB, yellow) and no (<45 dB) hearing loss thresholds. **** p<0.0001 by two-way ANOVA followed by All Pairwise Multiple Comparison Procedures (Holm-Sidak method)

Conclusions

- RW injections of GJB2-GT into conditional *Gjb2*-mutant mouse inner ears lead to improvement of hearing thresholds as early as 3 weeks post-injection in a dose dependent manner.
- GJB2-GT offers broad coverage of *Gjb2*-expressing cells of the inner ear in both mouse and NHPs. No transduction was found in inner hair cells, and no hair cell loss or ototoxicity were associated with GJB2-GT.
- GJB2-GT allows to efficiently and safely target the cells that naturally express GJB2 in the cochlea with both tropism and levels compatible with therapeutic intervention in human.
- Biodistribution studies in NHP shows minimal level of VCN in peripheral tissues that decreases overtime. Strong detection of vector of DNA and transgene derived mRNA is observed at the injection site (temporal bone) and transgene mRNA is almost undetectable in VCN positive peripheral tissues and contralateral ears, demonstrating very low ectopic Cx26 expression outside the inner ear.
- GJB2-GT is progressing as planned into IND/CTA enabling studies.