Safety and efficacy of GJB2-GT, an adeno-associated vector-based gene therapy treatment candidate for the autosomal recessive non-syndromic deafness 1A (DFNB1A)

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Abstract

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 neuroepithelium, fibrocytes, 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. **Here, we have developed GJB2-GT, an AAV vector for DNFB1A with proprietary cis-regulatory elements for a safe and directed expression of the transgene in target tissues *in vivo*.** Early tolerability and biodistribution studies for GJB2-GT or GJB2-Flag-GT were conducted in mice and Non-Human Primates (NHP) after injections through the round window (RW). **Intracochlear injection in both species resulted in transgene expression in most cells that naturally express Gjb2 along the tonotopic axis of the cochlea, with good local and systemic tolerability.** In all analyzed cochleae, no sensory hair cell expressed the transgene, confirming the specificity of the expression cassette. Importantly, GJB2-GT administration in NHP were performed using the surgical procedure and injection device intended for clinical use. **Long-term study conducted in wild-type mice demonstrated good safety with no impact on auditory function up to 1 year.** Moreover, histological analysis showed **long-term transgene expression and maintenance of the cochlear cytoarchitecture**. Additionally, high dose intravenous injection of GJB2-GT in mice did not induce any adverse events or changes in behavior and vital functions, and did not cause increase in hepatic damage or in inflammatory biomarkers. To circumvent embryonic mouse lethality caused by complete loss of Gjb2 expression, **a conditional model was generated, resulting in a biallelic Gjb2 inactivation mimicking the most common forms of DFNB1A and severe/profound hearing loss.** Intracochlear injections of GJB2-GT led to improvement of hearing thresholds as early as 3 weeks post-injection in a dose-dependent manner, with statistically significant results across multiple frequencies. Microscopic examination of injected cochlea confirmed that **GJB2-GT efficiently restored Cx26 expression in the sensory epithelium**. Current dose-response experiments confirm that **intracochlear GJB2-GT injection is safe over a wide range of administered doses and provides significant therapeutic benefit in our DFNB1A mouse model**. Altogether, these data support GJB2-GT development to restore physiological hearing in DFNB1A patients.

1. Natural history of our conditional mouse model *Gjb2*$^{cKO1/cKO1}$ mimicking DFNB1A physiopathology

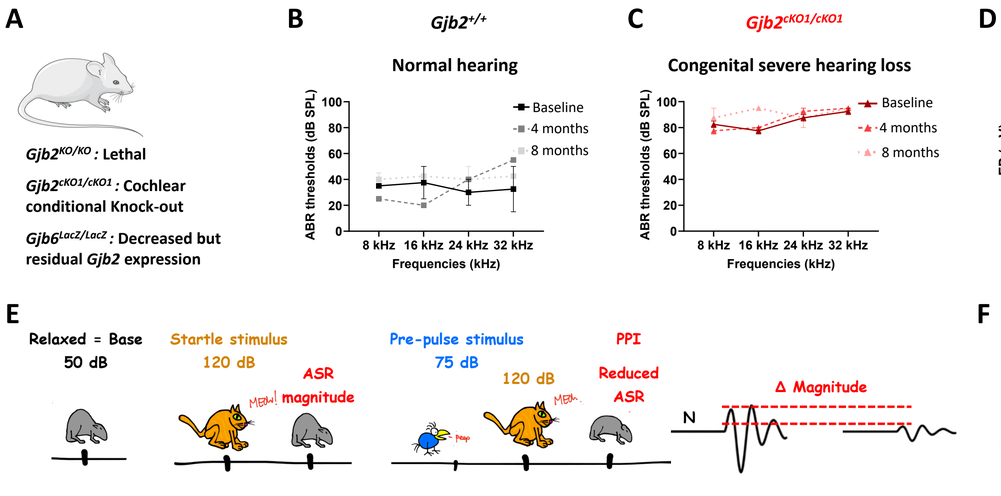


Figure 1 : A. Our engineered *Gjb2*$^{cKO1/cKO1}$ mutant mouse model is a suitable tool to study DFNB1A physiopathology and validate proof of concept (POC) studies, as the homozygosity for the *Gjb2*$^{KO/KO}$ allele has proven lethal in other mouse models during the embryonic development. Moreover, POC studies using the *Gjb6* mutant mouse model only manifest reduced *Gjb2* expression levels in the cochlea, instead of a full KO like in our *Gjb2*$^{cKO1/cKO1}$ mouse model. **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 (**B**) and do not present age-related hearing loss due to the absence of Cdh23AHL allele in the genetic background. In comparison, *Gjb2*$^{cKO1/cKO1}$ mutant mice demonstrate congenital severe hearing loss with Auditory brainstem response (ABR) thresholds above 80 dB (**C**). **Our ongoing efficacy studies currently focus on the congenital form. D.** Endocochlear Potential values of 1 month-old mice Gjb2$^{+/+}$ vs *Gjb2*$^{cKO1/cKO1}$. **Mutant mice of our model display a deficit in EP. E.** Principle of acoustic startle response (ASR) and pre-pulse inhibition (PPI) of ASR (Maze Engineers 2014). **Our mutant mice show reduced startle inhibition after PP.** N=Newton

2. Proprietary regulatory sequence allows specific expression of the transgene in target cells while efficiently de-targeting sensory hair cells

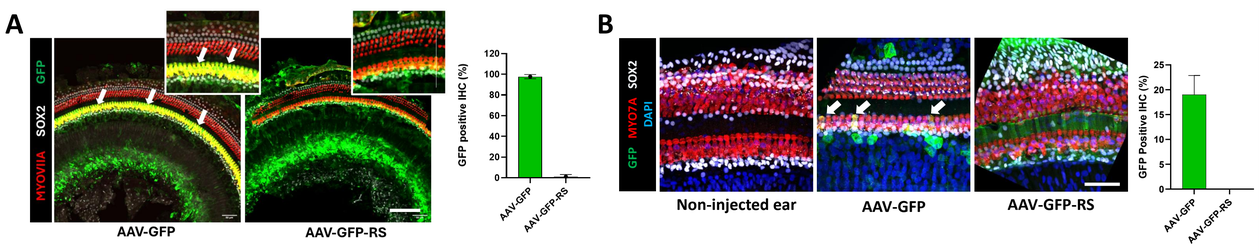
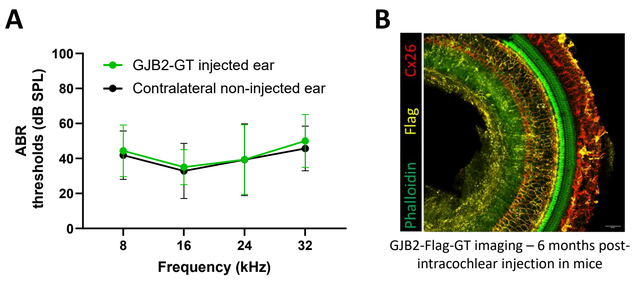


Figure 2 : A,B. Whole mount imaging of organ of Corti from mice (**A**) and NHP (B) injected with AAV-GFP or AAV-GFP-RS. Arrows indicate transduced inner hair cells (IHCs). Scale bar 40 and 50 µm, respectively. Quantification graphs of GFP+ IHCs in the mouse (**D**) and in the NHP (**E**) are shown. **Sensorion's proprietary regulatory sequence (RS) effectively abolishes transgene expression in sensory hair cells of the cochlea.**

3. Intracochlear and systemic injection of GJB2-GT is well tolerated in mice

1-year exploratory safety study : intracochlear injection



GJB2-Flag-GT imaging – 6 months post-intracochlear injection in mice

Acute toxicity study : High dose IV injection

- Experiment performed in preparation of upcoming GLP toxicity study in mice after IV injection
- GJB2-GT does not interfere with normal growth and does not lead to elevated transaminase levels 4 and 8 weeks after injection
- Behavioral evaluation - Functional Observation Battery, exploratory behavior (video tracking), 3 and 7 weeks after injection: no findings

Figure 3 : A. WT mice were injected with the indicated vectors through the RWM. ABR thresholds were measured up to 1 year after vector administration. **A. 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. Injected cochleae displayed normal cytohistology. No transgene expression was detected in hair cells, de-targeting of hair cells efficient in the long term.**

4. Our clinical candidate SENS-601 exhibits similar efficacy to GJB2-GT

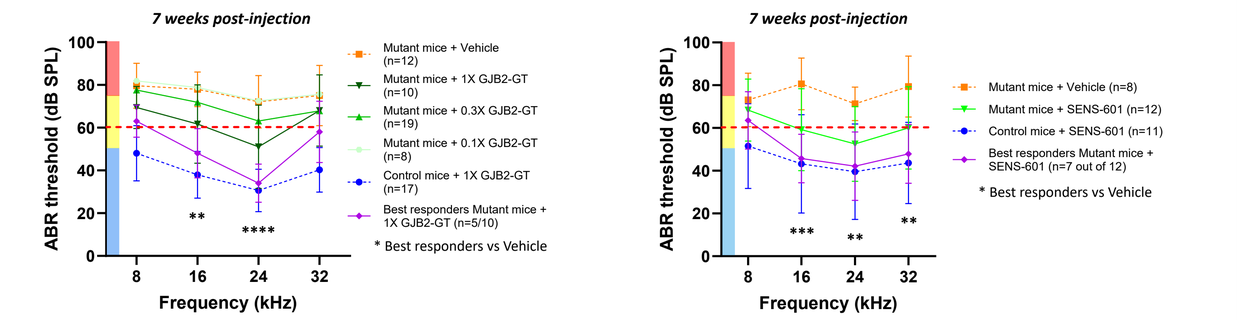


Figure 4 : A. GJB2-GT dose study. Average ABR recordings for GJB2-GT at doses 0.1X to 1X (light to dark green) or vehicle (dotted orange) obtained from GJB2-GT-injected *Gjb2*$^{cKO1/cKO1}$ mutant mice and GJB2-GT-injected *Gjb2*$^{+/+}$ control mice (blue) 7 weeks after injection. **B. SENS-601 efficacy study.** Average ABR recordings for SENS-601 at dose 1X (green) or vehicle (dotted orange) obtained from SENS-601-injected *Gjb2*$^{cKO1/cKO1}$ mutant mice and SENS-601-injected *Gjb2*$^{+/+}$ control mice (blue) 7 weeks after injection. Purple traces represent average ABR measurements for the 5 (**A GJB2-GT**) or 7 (**B SENS-601**) best responder animals, respectively, presenting average ABR thresholds across tested frequencies ≤ 60 dB at dose 1X. **A 1X dose of either GJB2-GT (A) or SENS-601 (B) 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, blue) hearing loss thresholds. **** p<0.0001 by two-way ANOVA followed by All Pairwise Multiple Comparison Procedures (Holm-Sidak method). **SENS-601, our future drug candidate, shows efficacy similar to GJB2-GT (R&D batch).**

5. Evaluation of the transduction efficiency of GJB2-GT in cochleae of *Gjb2*$^{cKO1/cKO1}$ mutant mice after a RW injection

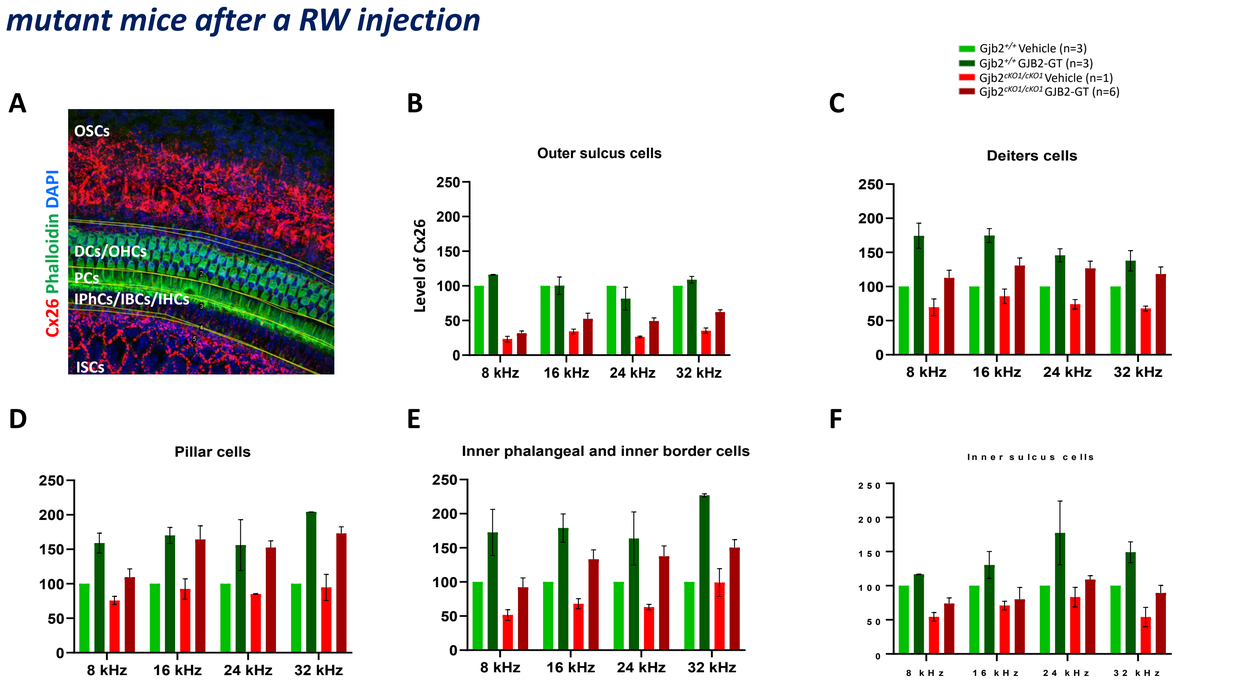


Figure 5 : Preliminary quantification of GJB2-GT expression in the different areas of supporting cells within the organ of Corti. A. After being micro-dissected, organ of Corti were stained for Cx26 (red) and counterstained with phalloidin (green) and DAPI (blue) and processed in whole mount. **B-F.** Quantification of Cx26 staining in different supporting cell type areas. All values are normalized toward the vehicle-injected *Gjb2*$^{+/+}$ animals. Injection of GJB2-GT restored expression of Cx26 in Deiters' cells (**C**), Pillar cells (**D**), and Inner Phalangeal and Inner Border cells (**E**) and had no major effect on Cx26 expression in Inner Sulcus cells (**F**). OSC=outer sulcus cell, ISC=inner sulcus cell

6. Injected therapeutic drug SENS-601 is specifically directed to target cells in NHP and is well tolerated

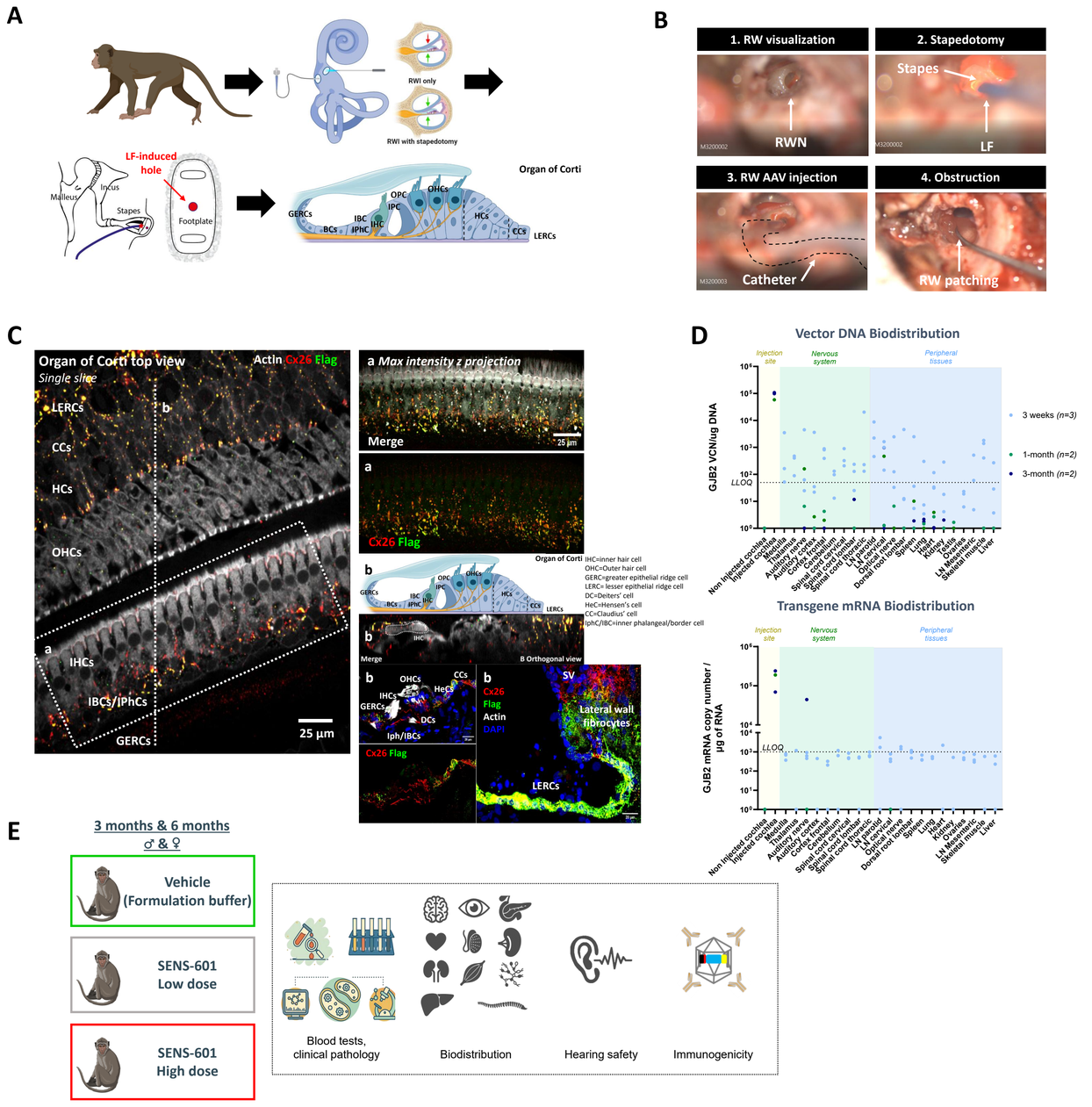


Figure 6 : A, B. GJB2-Flag-GT administration was performed in NHP using the RW approach (**B1,** RWN niche) and the medical injection device designed for the current use in Sensorion's gene therapy clinical trials. Briefly, a transcanal approach is performed. After visualization of the footplate of the stapes (**B1**), a stapedotomy is performed using a laser fiber (LF) (**B2**). An in-house designed catheter (**B3**) connected to the medical injection device is inserted in the RWM and the intracochlear administration performed via catheter. **C.** Three weeks post-injection, NHPs were euthanized, temporal bones collected and fixed in PFA. After decalcification, 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 (**Ca**) as well as cochlea cryosections (**Cb**) are shown to illustrate the cytoarchitecture of the tissue. **Using the surgery route and injection device designed for human, GJB2-GT-Flag vector administration results in correct expression of Cx26 at the cell membrane of target cells with no expression in hair cells. D.** Biodistribution of GJB2 vector genome (upper panel) and transgene mRNA (lower panel) in the cochlea and peripheral tissues of GJB2-GT-injected NHP. Biodistribution studies were conducted 3 weeks, 1 month and 3 months after vector administration. 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. The vast majority of the vector remains in injected ears, no dissemination observed in gonads, main organs, dorsal root ganglion (DRG). Overall, GJB2-GT intracochlear administration was well tolerated by the animals and did not induce any macroscopic/organ weight changes. There was no lab or clinical findings over the course of the study. **E.** Design of ongoing GLP toxicity and biodistribution study and planned readouts. LLOQ=lower limit of quantification

Conclusions

- In our GJB2-GT program, combination of an AAV serotype with high tropism toward supporting cells and a specific regulatory element offers broad coverage of Gjb2-expressing cells of the inner ear in both mouse and NHP, with very limited off-target expression. No transgene expression was detected in sensory hair cells.
- GJB2-GT intracochlear administration efficiently restores hearing thresholds in a new conditional DFNB1A mouse model.
- Biodistribution studies in NHP show minimal level of VCN in peripheral tissues that decreases over time. Strong detection of vector DNA and transgene mRNA is observed at the injection site (temporal bone) and mRNA is almost undetectable in VCN-positive peripheral tissues and contralateral ears, demonstrating very low ectopic Cx26 expression outside the inner ear.
- SENS-601, the clinical candidate, reestablishes hearing thresholds efficiently and demonstrates an appropriate safety and efficacy profile to support its development for therapeutic use in DFNB1A patients. The program is progressing as planned with CTA submission in Q1 2026.