Safety, biodistribution and efficacy of SENS-601, an AAV-based gene therapy treatment candidate for the autosomal recessive non-syndromic deafness 1A (DFNB1A)

Guillaume Olivier[1], Christophe Tran Van BA[1], Sandra Pierredon1[1], Anne-Gabrielle Harrus[1], Lise Barrot[1], Audrey Broussy[1], Selma Dadak[1], Christine Le Bec[2], Anne-Valérie Héritier[3], Amrit Estivalet[3], Andrea Lelli[3], Muriel Sudres[3], Nicolas Michalski[3], Geraldine Petit[1], Rafik Boudra[1], Arnaud Giese[1], Christine Petit[3, 4], **Laurent Desire**[2] - 1 Sensorion, Montpellier, France, 2 Sensorion, Paris, France, 3 Université Paris Cité, Institut Pasteur, APHP, Inserm, Fondation Pour l'Audition, Institut de l'Audition, IHU reConnect, Paris, France, 4 Université Paris Cité, Institut Pasteur, AP-HP, Inserm, Fondation Pour l'Audition, Institut de l'Audition, IHU reConnect, Collège de France, Université Paris Science et Lettres, Paris, France – contact info: laurent.desire@sensorion-pharma.com

ANNUAL MEETING BOSTON, MA • May 11-15, 2026

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), the main connexin in the cochlea, are involved in 50% of congenital deafness and are mostly associated with autosomal recessive non-syndromic deafness DFNB1A. Over 130 distinct GJB2 mutations associated with hearing loss have been identified in clinical settings. Treatment options primarily consist of hearing aids and cochlear implants, the effectiveness of which remains unsatisfactory. 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,,through the formation of heteromeric or heterotypic gap junctions, is essential for energy supply and ionic balance in the cochlea, which are essential for the proper functioning of sensory hair cells. Recent clinical trials are demonstrating promising hearing recovery in patients treated with gene therapy strategies for autosomal recessive forms of deafness.

Here, we have developed SENS-601, an AAV vector for DNFB1A with proprietary cis-regulatory elements for a safe and targeted expression of the transgene in vivo. Long-term studies were conducted in wildtype mice and Non Human Primates (NHP) to demonstrate persistence of transgene expression with no impact on auditory function. To evaluate the local tolerability and safety of SENS-601, a combined 3- and 6-month GLP toxicology and biodistribution study has been conducted in NHP, following a single intracochlear injection administered, using the surgical procedure and injection device intended for clinical use. For efficacy and dose-ranging studies, a conditional knock-out mouse model was generated to circumvent embryonic mouse lethality caused by complete loss of Gjb2 expression, resulting in a biallelic Gjb2 inactivation mimicking the most common forms and pathophysiology of DFNB1A with severe/profound congenital hearing loss.

1. A proprietary regulatory sequence in SENS-601 restricts transgene expression to target cells in the cochlea with no ectopic expression in sensory hair cells in mice and NHP

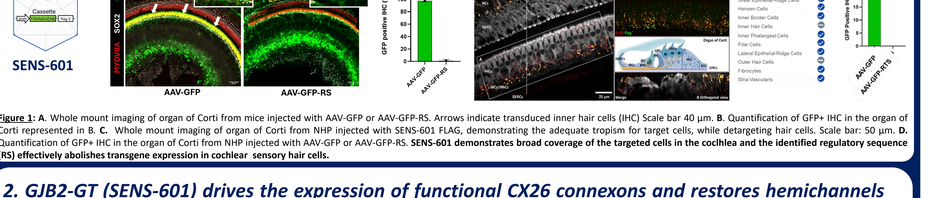


Figure 1: A. Whole mount imaging of organ of Corti from mice injected with AAV-GFP or AAV-GFP-RS. Arrows indicate transduced inner hair cells (IHC) Scale bar 40 µm. **B.** Quantification of GFP+ IHC in the organ of Corti represented in B. **C.** Whole mount imaging of organ of Corti from NHP injected with SENS-601 FLAG, demonstrating the adequate tropism for target cells, while detargeting hair cells. Scale bar: 50 µm. **D.** Quantification of GFP+ IHC in the organ of Corti from NHP injected with AAV-GFP or AAV-GFP-RS. **SENS-601 demonstrates broad coverage of the targeted cells in the cochlea and the identified regulatory sequence (RS) effectively abolishes transgene expression in cochlear sensory hair cells.**

2. GJB2-GT (SENS-601) drives the expression of functional CX26 connexons and restores hemichannels functions including in contexts associated with most common human CX26 pathogenic variants

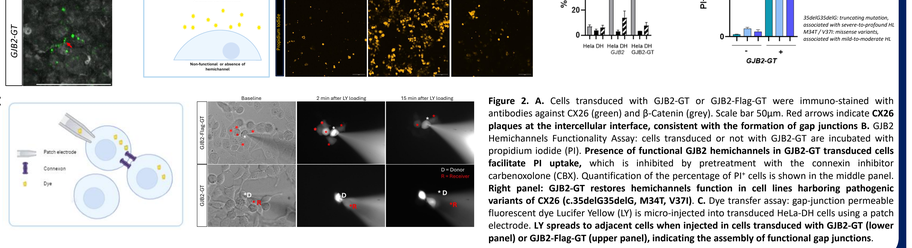


Figure 2. A. Cells transduced with GJB2-GT or GJB2-Flag-GT were immuno-stained with antibodies against CX26 (green) and β-Catenin (grey). Scale bar 50µm. Red arrows indicate **CX26 plaques at the intercellular interface, consistent with the formation of gap junctions B.** GJB2 Hemichannels Functionality Assay: cells transduced or not with GJB2-GT are incubated with propidium iodide (PI). **Presence of functional GJB2 hemichannels in GJB2-GT transduced cells facilitate PI uptake,** which is inhibited by pretreatment with the connexin inhibitor carbenoxolone (CBX). Quantification of the percentage of PI' cells is shown in the middle panel. **Right panel: GJB2-GT restores hemichannels function in cell lines harboring pathogenic variants of CX26 (c.35delG35delG, M34T, V37I). 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 spreads to adjacent cells when injected in cells transduced with GJB2-GT (lower panel) or GJB2-Flag-GT (upper panel), indicating the assembly of functional gap junctions.**

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

1-year exploratory safety study: intracochlear injection

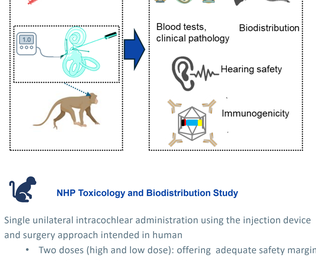
GT-GJB2-Flag imaging – 6 months post intracochlear injection in mice

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 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

Figure 3: A. WT mice were injected with the indicated vectors through the RWM. Auditory brainstem response (ABR) were measured up to 1 year 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. Injected cochleae displayed normal histology. No ectopic transgene expression was detected in hair cells**

4. SENS-601 is safe and well tolerated in mice and NHP (combined 3- and 6-month GLP toxicology and biodistribution studies)



Mouse Toxicology and BiodistributionStudy

Single intravenous administration
- Two doses (high and low dose): offering adequate safety margin to cover unintentional systemic exposure

- Mortality: No unscheduled death related to SENS-601
- Clinical observations: No SENS-601-related clinical signs
- Body Weights and Body Weight Gains: No SENS-601-related effect
- Clinical pathology: slight changes on one parameter ,not associated with pathology signs and considered non adverse
- Histology: No SENS-601-related observations
- Dose-related quantification of SENS-601 DNA, with limited tissue exposure

NHP Toxicology and Biodistribution Study

Single unilateral intracochlear administration using the injection device and surgery approach intended in human
- Two doses (high and low dose): offering adequate safety margin according to Regulatory expectations

- Mortality: No unscheduled death related to SENS-601
- Hematology / coagulation / urinalysis: No SENS-601-related changes
- Clinical pathology: minor non-adverse change in one parameter
- Histology:
  Minimal to mild observations, as expected from local surgery procedure and in the context of inner ear gene therapy
- Biodistribution and shedding:
  SENS-601 DNA detection in expected tissues in the context of an intra-cochlear injection and is dose-related
  Shedding decreasing over time
- Mild SENS-601-related immunoreactivity resolving over time

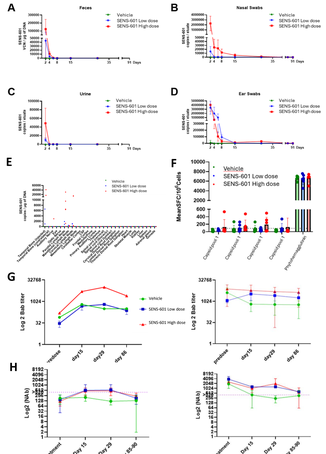


Figure 4: Results at 3 months timepoint in NHP study. A-D. SENS-601 shedding in feces (A), nasal swabs (B), urine (C), ear swabs (D). **E. SENS-601 DNA was mainly distributed in all analysed ipsilateral temporal bone (injection site),** draining (cervical and parotid) lymph nodes surrounding the injection site, sporadically in auditory nerve samples and mandibular lymph nodes. No SENS-601 is detected and/or quantified in contralateral temporal bones or main organs. **SENS-601 mRNA was quantified in all treated temporal bone samples (not shown). F. SENS-601 does not induce a robust or widespread cellular immune response against the capsid or transgene. G. Anti-SENS-601 binding antibodies. H. Anti-SENS-601 neutralizing antibodies.** Left graph: animals with low pre-treatment seropositivity. Right: animals with high pre-treatment seropositivity. **For animals with low pre-treatment titers, binding and neutralizing antibodies titers increased post-treatment, before declining by Day 85-90.**

5. Efficacy and dose studies in Gjb2$^{cKO1/cKO1}$ mutant mice

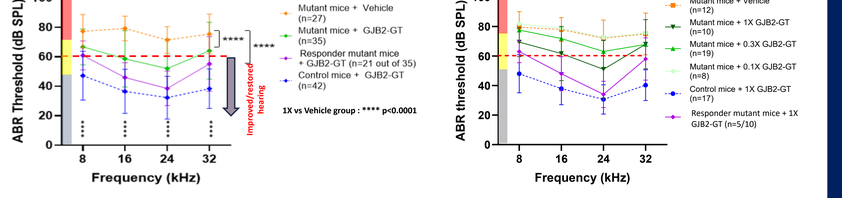


Figure 5: A. Efficacy study. ABR recordings at 7 weeks post-administration for GJB2-GT injected (green) or vehicle (orange) *Gjb2*$^{cKO1/cKO1}$ mutant mice and for GJB2-GT injected *Gjb2*$^{+/+}$ control mice (average – blue) 7 weeks after intracochlear injection. Purple line represent average ABR measurements for the responder animals, presenting average ABR thresholds across tested frequencies ≤ 60 dB (red dotted line). Data are pooled from independent studies involving R&D batches and the batch used for the GLP toxicology studies. **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 study.** Average ABR recordings for GJB2-GT 1X to 0.3X (dark to light green) or vehicle (dotted orange) injected *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 5 responder animals, presenting average ABR thresholds across tested frequencies ≤ 60 dB at dose 1X. **A 1X 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)

6. SENS-601 efficiently restores Cx26 expression and Cx26 network continuum in the sensory epithelium, which correlates with significant ABR threshold restoration in Gjb2 CKO1 model

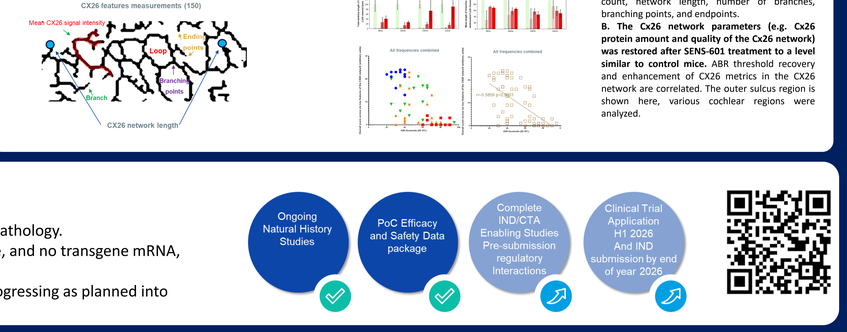


Figure 6: Analysis of Cx26 network using IA-based image analysis across numerous representative features of the Cx26 network within the cochlea. A. Cx26 network complexity was analyzed after thresholding and skeletonization in a model based on the analysis of ~600 images and ~ 150 analyzed features/image, then reduced to statistically significant essential features characterizing the CX26 network including loop count, network length, number of branches, branching points, and endpoints.
B. The Cx26 network parameters (e.g. Cx26 protein amount and quality of the Cx26 network) was restored after SENS-601 treatment to a level similar to control mice. ABR threshold recovery and enhancement of CX26 metrics in the CX26 network are correlated. The outer sulcus region is shown here, various cochlear regions were analyzed.

Conclusions

- SENS-601 offers broad coverage and selectivity for *Gjb2*-expressing cells of the inner ear.
- SENS-601 efficiently restores hearing threshold in a conditional DFNB1A mouse model highly relevant for the pathology.
- Biodistribution studies in NHP shows minimal level of vector DNA in peripheral tissues that decreases overtime, and no transgene mRNA, demonstrating very low ectopic Cx26 expression outside the inner ear
- GJB2-GT safety and efficacy profile supports its development for therapeutic use in humans, the program is progressing as planned into IND/CTA enabling studies.

Ongoing Natural History Studies

PoC Efficacy and Safety Data package

Complete IND/CTA Enabling Studies Pre-submission regulatory Interactions

Clinical Trial Application H1 2026 And IND submission by end of year 2026