SENS-601, a Novel Adeno Associated Vector-Based Gene Therapy as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 1A (DFNB1A)

Presenting author (Laurent DESIRE), Guillaume Olivier[1], Christophe Tran Van Ba[1], Sandra Pierredon[1], Anne-Gabrielle Harrus[1], Lise Barrot[1], A Broussy[1], S Dadak[1], Carine Moigneu[1], Natacha Soudier[1], C Le Bec[1], Anne-Valérie Héritier[2], Amrit Singh-Estivalet[2], Charlène Joséphine[1], Charlène Vaux[1], Vincent Decossy[1], Andrea Lelli[2], Muriel Sudres[2], Nicolas Michalski[2], Geraldine Petit[1], Rafik Boudra[1], Arnaud Giese[1], Christine Petit[2,3], Laurent Desire[1]
[1]Sensorion, [2]Institut de l'Audition, [3]Collège de France – contact info: laurent.desire@sensorion-pharma.com

Abstract

SENS-601 is an adeno-associated virus-based gene therapy candidate developed for autosomal recessive non-syndromic deafness 1A (DFNB1A), caused by pathogenic variants in the GJB2 gene encoding the connexin 26 (CX26) protein. DFNB1A accounts for upt to 50% of congenital, severe-to-profound, autosomal recessive non-syndromic hearing loss, for which current therapeutic options, hearing aids and cochlear implants, remain suboptimal. We engineered SENS-601 with proprietary cis-regulatory elements designed to enable safe and targeted transgene expression in supporting cells, the native site of GJB2 expression, while avoiding sensory hair cells where GJB2 is not expressed. In vitro, SENS-601 efficiently transduced human cells, restored Cx26 localization at the plasma membrane, and allowed functional gap junction plaques, including in the context of known human pathogenic GJB2 variants. In vivo, cochlear delivery in mice and non-human primates resulted in selective transgene expression along the tonotopic axis of the cochlea in cells that naturally express Gjb2, with no detectable expression in sensory hair cells and good local and systemic tolerability. Long-term studies in wild-type mice showed persistent transgene expression without adverse effects on hearing or cochlear cytoarchitecture. We completed a GLP-compliant 3- and 6-month toxicology and biodistribution study in non-human primates, performed by single intracochlear administration using the surgical procedure and delivery device intended for clinical use. The study showed no treatment-related adverse findings, and minimal off-target biodistribution and vector shedding. No robust or widespread humoral or cellular immune response to the capsid or transgene was observed after intracochlear dosing. To assess efficacy, we generated conditional Gjb2-deficient mouse models recapitulating the most common forms of DFNB1A-associated severe congenital hearing loss. Intracochlear administration of SENS-601 produced dose-dependent and statistically significant restored hearing thresholds as early as 3 weeks post-injection, across multiple frequencies and confirmed across various additional readouts. Histological analyses confirmed restoration of CX26 expression and reconstitution of the cochlear gap junction network in the sensory epithelium. Together, our comprehensive safety and efficacy data package supports our upcoming SENS-601 first in human testing, as a promising therapeutic candidate to restore physiological hearing in DFNB1A patients

1. SENS-601 provides selective CX26 transgene expression in target cells in the cochlea, with built-in hair cell transgene silencing

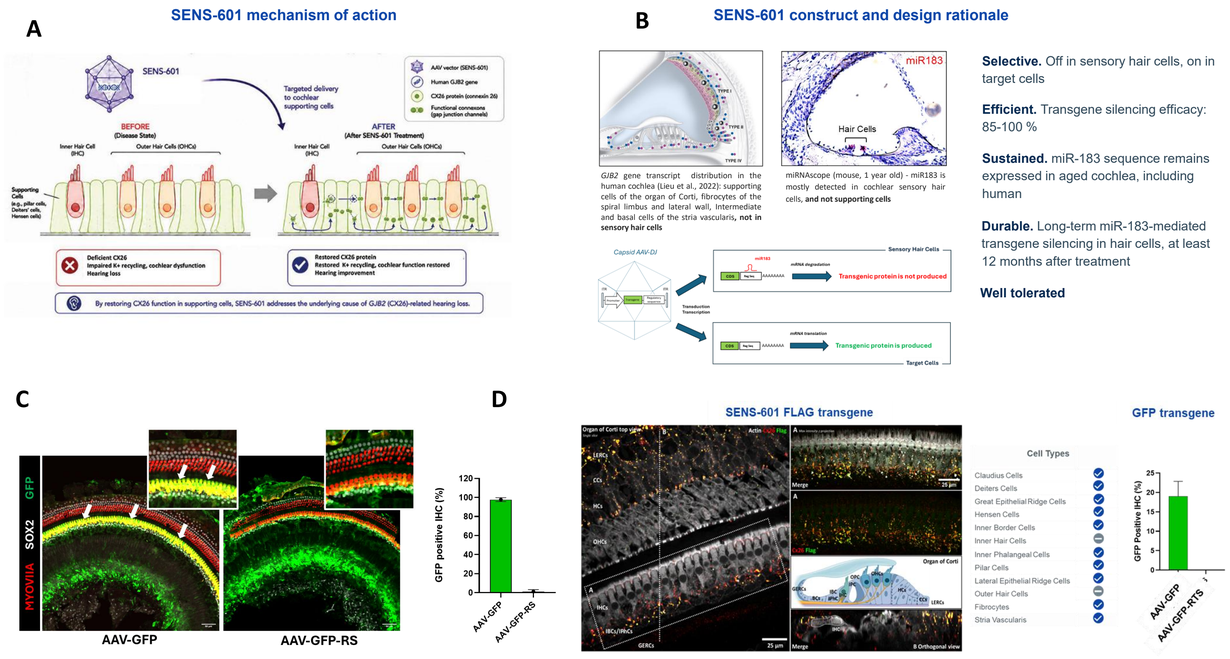


Figure 1: A SENS-601 aims to restore physiological hearing by restoring CX26 protein, thereby restoring CX26-containing gap junctions and associated cochlear homeostasis in DFNB1A patients. B. A built-in miR-183 switch silences the transgene in hair cells and leaves it active in target supporting cells, where CX26 is expressed in the cochlea. **C.** Whole mount imaging of organ of Corti from mice injected with AAV-GFP or AAV-GFP-RS (proprietary miR183 target sequence). Arrows indicate transduced inner hair cells (IHC) Scale bar 40 µm. Quantification of GFP+ IHC in the organ of Corti is presented. **D.** Whole mount imaging of organ of Corti from NHP injected with SENS-601 FLAG, demonstrating the adequate tropism for target cells, with no expression in hair cells. Scale bar: 50 µm. Quantification of GFP+ IHC in the organ of Corti from NHP injected with AAV-GFP or AAV-GFP-RS is presented. **SENS-601 demonstrates broad coverage of the targeted supporting cells in the cochlea and the regulatory sequence 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

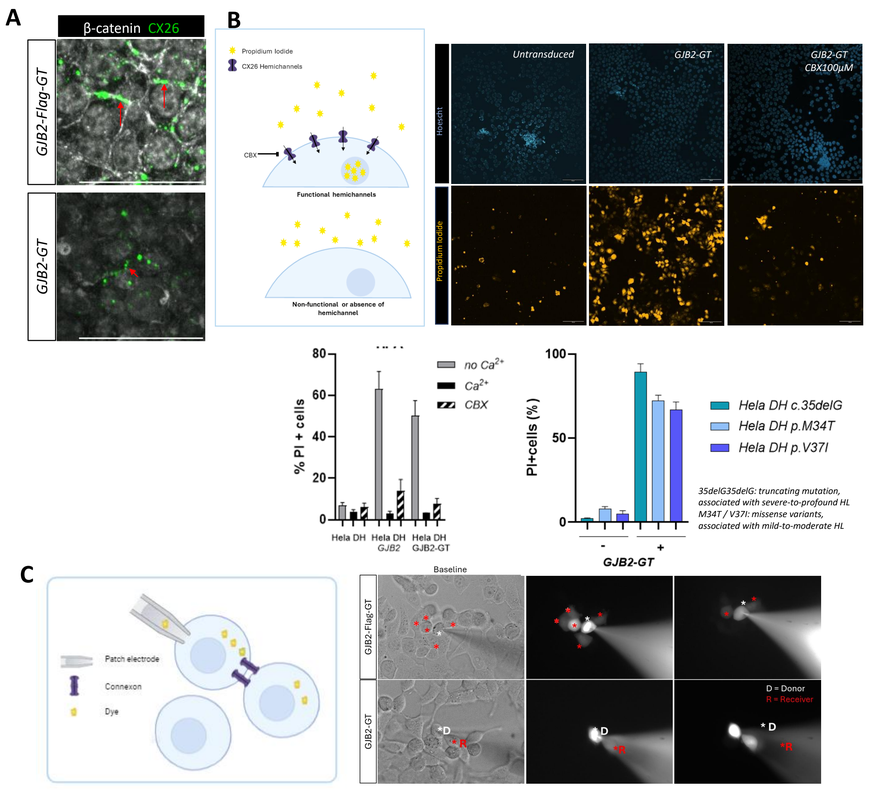


Figure 2. A. Cells transduced with GJB2-GT or GJB2-Flag-GT were immuno-stained with antibodies against CX26 (green) and β-Catenin (grey). Scale bar 50µm. Red arrows indicate **CX26 plaques at the intercellular interface, consistent with the formation of gap junctions B.** GJB2 Hemichannels Functionality 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

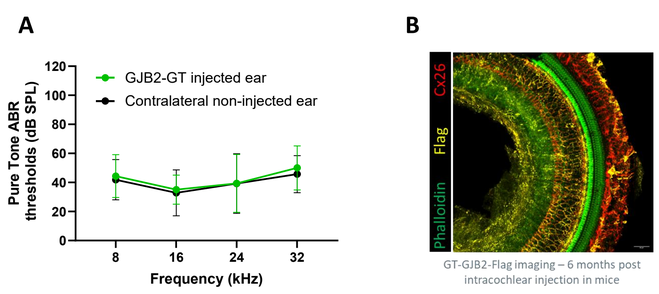
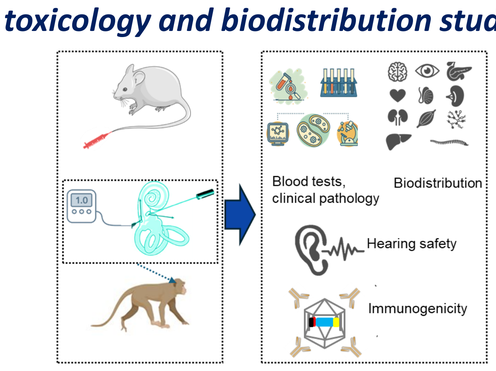


Figure 3: A. WT mice were injected with the indicated vectors through the RWM. Auditory brainstem response (ABR) were measured up to 1 year after vector administration. **GJB2-GT injected animals display normal ABR thresholds, consistent with good local tolerability of the vector B**. Whole-mount imaging 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, still detectable at 6 months, mainly at the injection site (tail) and in the liver (highest level); DNA in blood up to Week 5

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

- 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 confined to the injected ear and draining lymph nodes and is dose-related
  Shedding decreasing over time
- Mild SENS-601-related immunoreactivity resolving over time

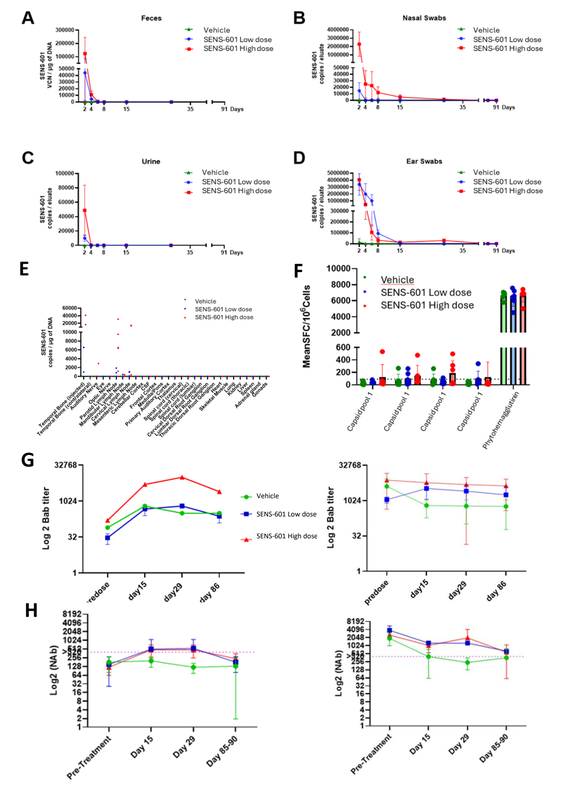


Figure 4: Results at 3 months timepoint in NHP study. A-D. SENS-601 shedding in feces (A), nasal swabs (B), urine (C), ear swabs (D). **E. SENS-601 DNA was mainly distributed in all analysed ipsilateral temporal bone (injection site),** draining (cervical and parotid) lymph nodes surrounding the injection 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 with congenital severe-to-profound hearing loss, mimicking DFNB1A disease

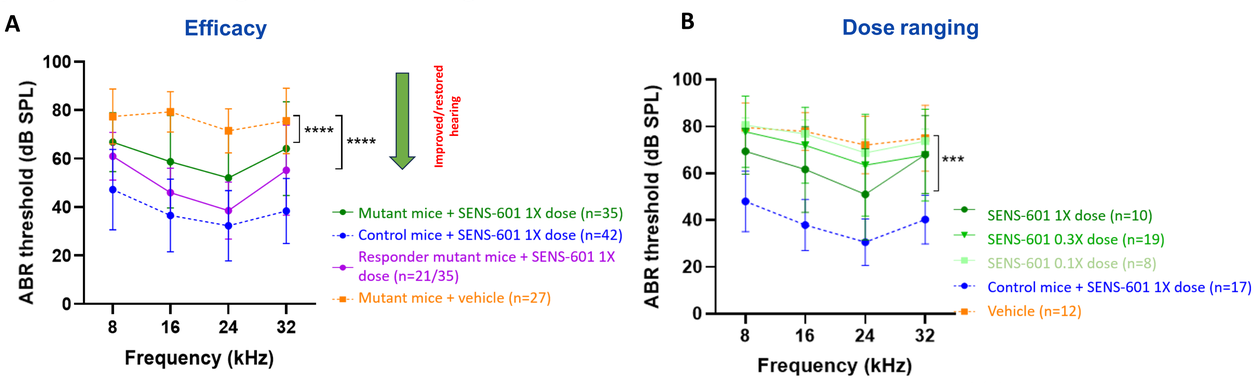


Figure 5: A. Efficacy study. ABR recordings at 7 weeks post-administration for SENS-601 injected (green) or vehicle (orange) $Gjb2^{cKO1/cKO1}$ mutant mice and for SENS-601 injected $Gjb2^{+/+}$ control mice (average – blue). Purple line represent average ABR measurements for the responder animals, presenting average ABR thresholds across at least one tested frequency ≤ 60 dB. Data are pooled from independent studies involving R&D batches and the batch used for the GLP toxicology studies. **SENS-601 induced a statistically significant hearing recovery after injection in $Gjb2^{cKO1/cKO1}$ mutant mice. B, Dose study.** Average ABR recordings for SENS-601 1X to 0.3X (dark to light green) or vehicle (dotted orange) injected $Gjb2^{cKO1/cKO1}$ mutant mice and for SENS-601 injected $Gjb2^{+/+}$ control mice (blue) 7 weeks (D) after injection. **A 1X dose of SENS-601 is sufficient to induce a statistically significant hearing recovery after injection in $Gjb2^{cKO1/cKO1}$ mutant mice.** **** p<0.0001; *** p<0.001 two-way ANOVA multiple comparisons followed by Tukey test

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 mutant mice

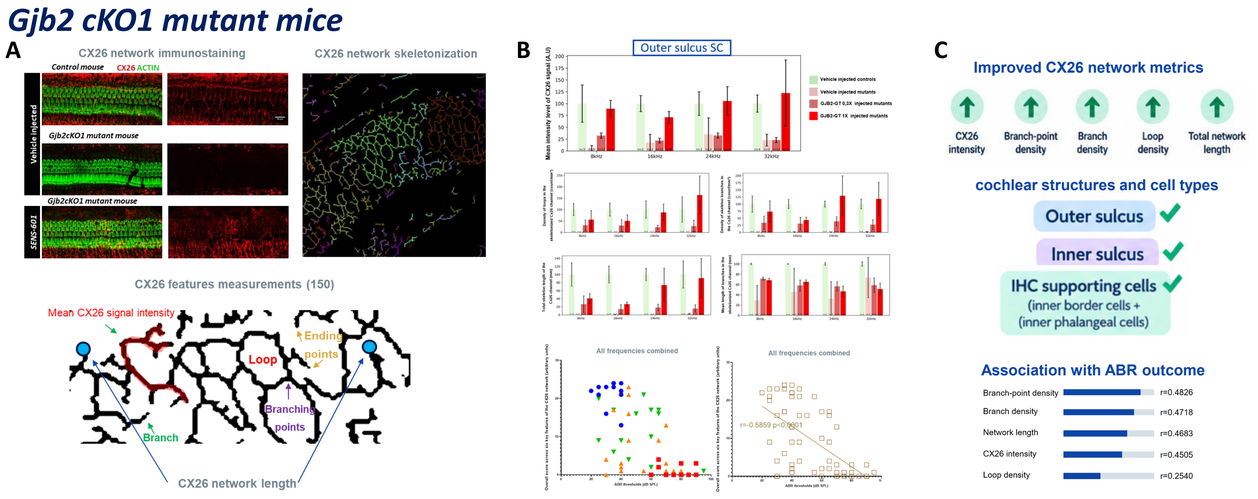


Figure 6: Analysis of Cx26 network using IA-based image analysis across numerous representative metrics of the Cx26 network within the cochlea. A. Cx26 network complexity was analyzed after thresholding and skeletonization in a model based on the analysis of ~600 images and ~ 150 analyzed features/image, then reduced to statistically significant essential metrics 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. **C. CX26 network metrics are improved in multiple cochlear structures and cell type.** Statistical association with ABR outcome is shown. Associations are significant: p<0.0001; loop density p=0.0135

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 Phase I/II trial.

