Preclinical development of SENS-501 as a treatment for the autosomal recessive non-syndromic deafness 9 (DFNB9) using an adeno associated vector-based gene therapy.

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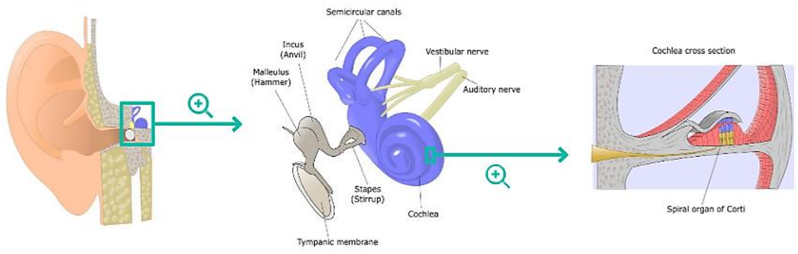
Abstract

Congenital sensorineural defects are one of the most severe forms of congenital impairment, heavily impacting the life of the patients and their ability to communicate with others. Among this vast family of communicating disorders, the non-syndromic autosomal recessive deafness 9 (DFNB9) is one of the most common forms of congenital deafness, accounting for up to 8% of cases. This severe-to-profound auditory neuropathy is caused by a biallelic loss of function in the *Otoferlin* gene (*OTOF*), which encodes for a calcium sensor protein involved in the neurotransmitter release at the presynaptic level between the inner sensory hair cells (IHC) and the spiral ganglion neurons. So far, the only medical solution is the cochlear implantation, which improves hearing to some degree, but still has a lot of limitations. To address this unmet medical need, we developed SENS-501, a dual AAV (adeno associated virus) hybrid approach using two different recombinant vectors each containing one half of the *OTOF* cDNA. This strategy was tested on congenitally deaf DFNB9 mutant mice by injecting SENS-501 in the inner ear through the round window membrane using different doses. The reversal of the deafness phenotype in our knock-out mouse model was evaluated through multiple auditory and behavioural tests. Auditory brainstem response (ABR) recordings showed significant lowering of the thresholds along the auditive spectrum after intra-cochlear injection, showing a durable improvement of hearing in a dose-responsive manner analyzed as early as three weeks post-injection and that lasted at least for one year. These mice were also submitted to a startle-test protocol experiment to confirm their ability to efficiently process sound similarly to wild-type mice. We were able to detect a restoration of their startle reflex ability when exposed to randomized sudden loud noises. We also evaluated the vestibular function of treated mice by analyzing their locomotion patterns in an open-field arena. Data indicated that our approach did not show any significant increase in circling behaviour when compared to control mice. We performed a similar set of experiments in non-human primates (NHP) after SENS-501 intracochlear injections using a surgical method and our proprietary medical delivery device envisioned in clinical trial. Surgery and SENS-501 did not affect ABR thresholds, similar to what was observed in mice. Immunohistochemistry experiments were performed in both mice and NHP, demonstrating effective recombination and selective expression in IHCs of the full-length therapeutic protein and its flag-tagged counterpart. A 3-month GLP toxicology and biodistribution study following a single intra-cochlear injection was conducted in NHP with two doses of SENS-501. Biodistribution data indicated that the vast majority of the vectors remained in ear structure that received the injection. SENS-501 shedding in fluids showed a limited dissemination of the vectors and all the shedding samples decreased over time. The two doses of SENS-501 were well tolerated and did not induce any macroscopic/organ weight changes or local/systemic microscopic findings, Therefore, our nonclinical pharmacology, biodistribution, and safety studies support the clinical development of SENS-501 for hearing loss due to genetic Otoferlin protein deficiency.



The inner ear is a good target for gene therapy

- Routine surgical access
- Isolated organ limiting off-target effects

Role of Otoferlin:

- OTOF is mainly expressed in cochlear inner hair cells
- Necessary for synaptic exocytosis at the ribbon synapse
- The remaining hearing chain is functional in DFNB9 young patients

1. Hybrid dual AAV vector strategy (SENS-501) and intracochlear injection

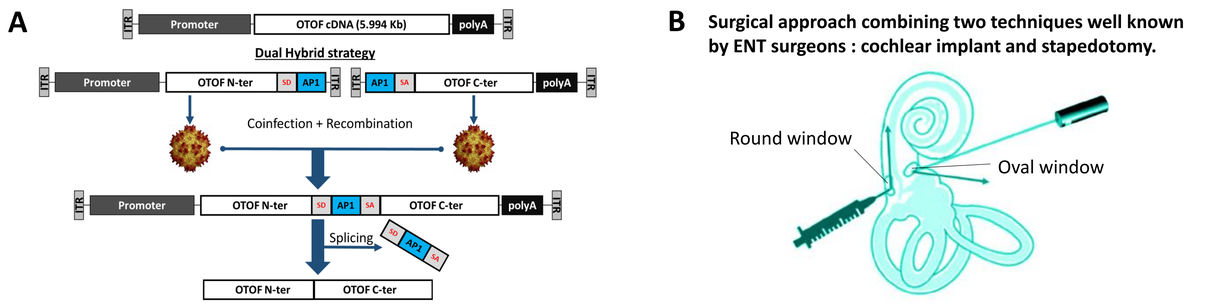


Figure 1: A. The size of the *OTOF* coding sequence largely exceeds AAV packaging capacity. To overcome this limitation, a dual AAV vector was designed, with *OTOF* coding sequence being split into two parts: i) a 5'-N-terminal (N-ter) vector, containing the N-terminal part of the AAV cassette, and ii) a 3'-C-terminal (C-ter) vector, containing the C-terminal part of the AAV cassette. After cells coinfection, DNA sequence alignment, homologous recombination via an overlapping sequence between the two cassettes, transcription and splicing of final mRNA, full-length Otoferlin protein is reconstituted. ITR: Inverted Terminal Repeat; SD/SA: Splicing Donor/Acceptor sequence; AP: Recombinogenic overlapping sequence **B.** Surgical approach optimized in the NHP GLP tox studies and envisioned in human that consists in injecting our therapeutic product SENS-501 in the round window with a combination of a stapedotomy procedure (Oval window) in order to alleviate the overpressure in the inner ear, avoid the backflow from the injection site and assure a homogenous transduction rate along the tonotopic axis of the cochlea.

2. Full-length Otoferlin protein is effectively reconstituted in vitro and in vivo

- *RT-PCR and Western-blot showed efficient recombination and splicing in HEK-293 cells*

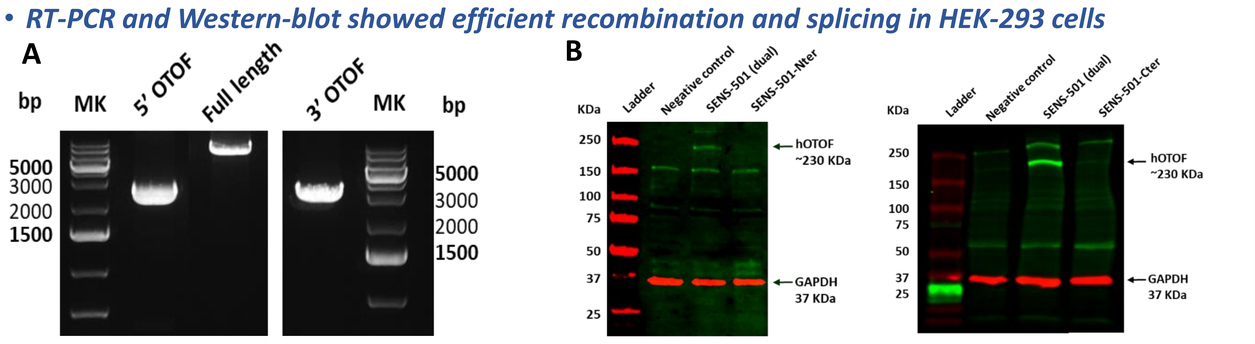


Figure 2: HEK-293 cells were coinfected with dual AAV vectors containing *Otoferlin* N-ter and C-ter sequences respectively (ratio 1:1).
A. RT-PCR on cells lysates show **highly efficient DNA recombination with full length mRNA amplification** and **B. highly efficient splicing with full-length Otoferlin protein expression** 3 days after coinfection. Western blot used an anti-Otof antibody directed against the N-ter (left) or C-ter (right) part of Otoferlin protein. **No Otoferlin protein expression is detected with single vectors N-ter or C-ter alone** (confirmed by Mass Spectometry).
CTL: control, KDa: kiloDalton, MOI: multiplicity of Infection, MK: ladder

3. SENS-501 cell-specific transduction, efficacy and stability of hearing restoration in an OTOF-KO (DFNB9) mouse model

- *Otoferlin is specifically expressed in inner hair cells* **in vivo** *after dual AAV vector injection*

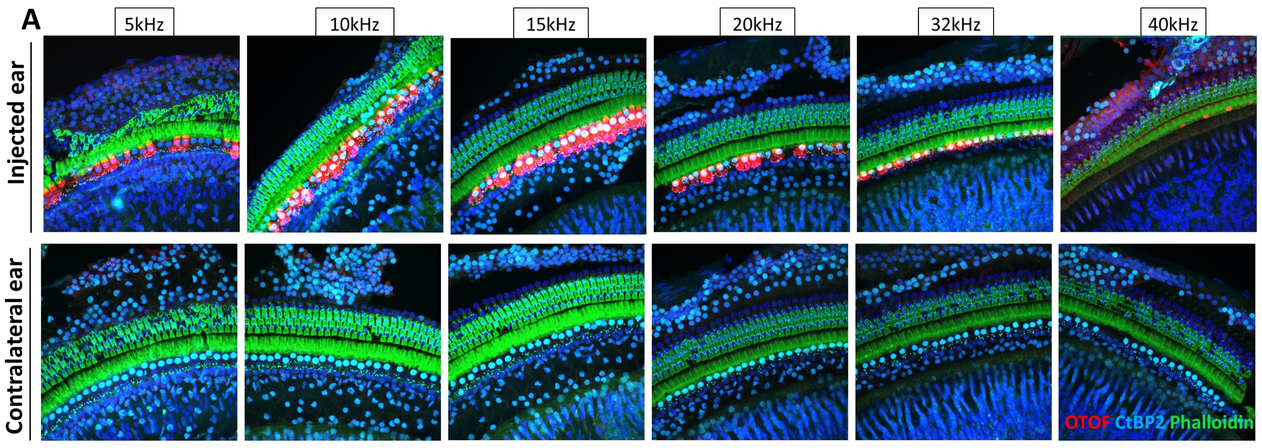


Figure 3: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing *Otoferlin* transgene cassette. 6 months after injection, cochlea were sampled and stained for Otoferlin. **A. *Otoferlin* is *de novo* expressed in inner hair cells in injected left ear** (A-top) *vs.* no expression in contralateral right ear (A-bottom). ***Otoferlin* is specifically expressed in inner hair cells (IHC) with no ectopic expression in outer hair cells (OHC) or other cochlear cell types**. **Otoferlin subcellular localization is comparable to endogenous protein. SENS-501 is locally well tolerated.**

- *Startle reflexes are restored in Otof*$^{KO/KO}$ *mutant mice and their exploratory behavior is not affected after injection of SENS-501*

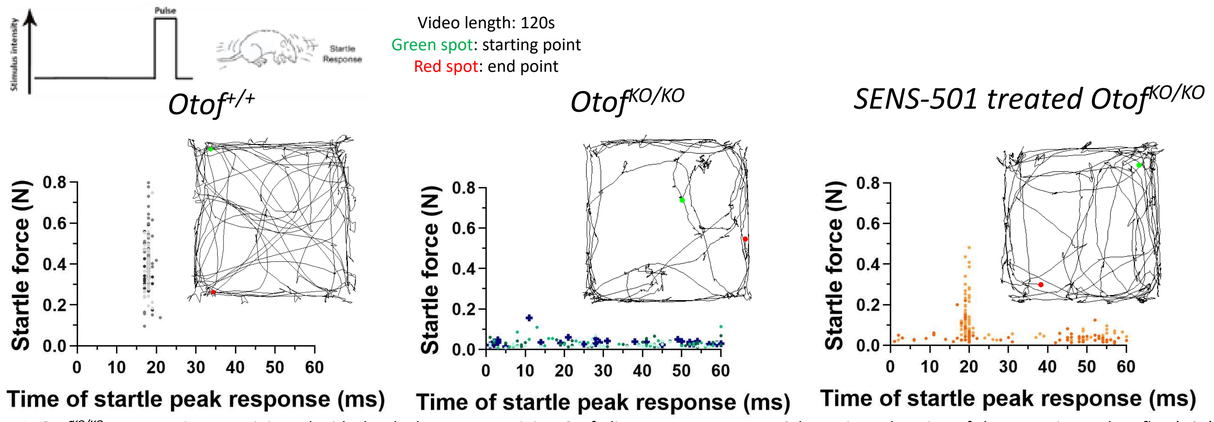
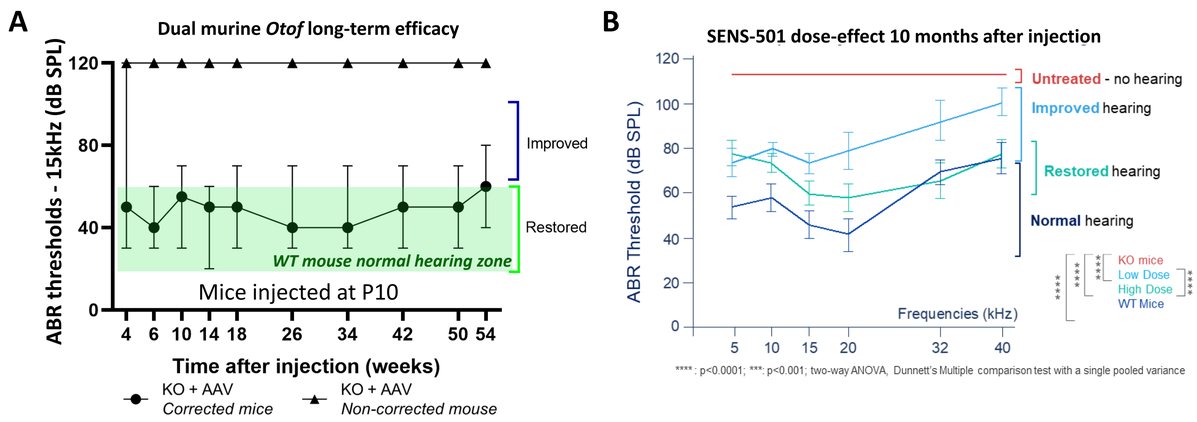
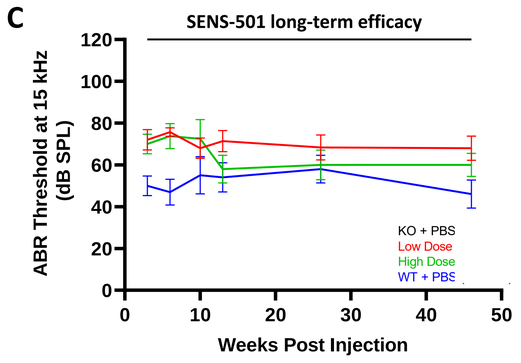


Figure 4: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing *Otoferlin* transgene cassette. Schematic explanation of the acoustic startle reflex (ASR) assay (adapted from Turner & Parrish, 2008 and Kraus KS *et al.)* is shown. Exploratory behavior and startle reflexes are shown for wild type mice (black) and *Otof*$^{KO/KO}$ mutant mice (blue) and SENS-501 treated *Otof*$^{KO/KO}$ mutant mice (orange) in response to a white noise stimulus (2-20kHz) at 115 dB.

4. SENS-501 leads to long-term hearing recovery in a translational model of Otoferlin deficiency: results from multiple efficacy studies





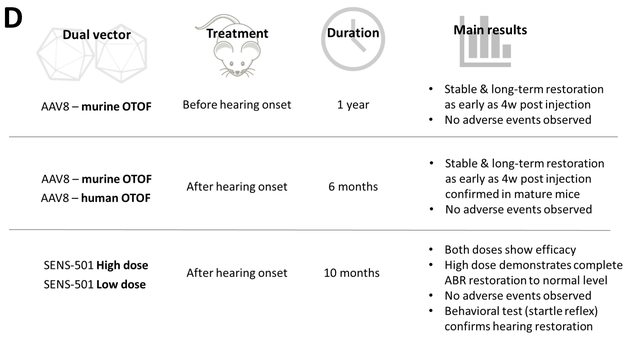
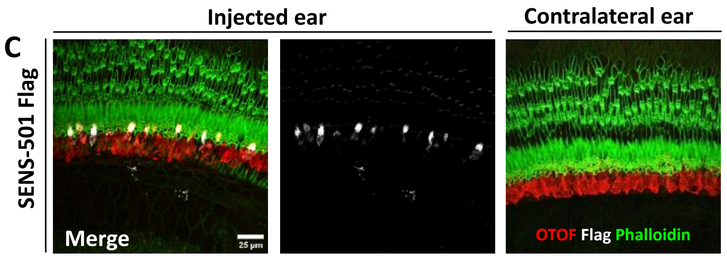


Figure 5: A. P10 old *Otof*$^{KO/KO}$ congenitally deaf mutant mice were injected with a dual vector containing the murine *otoferlin* transgene cassette (n=8). Auditory Brainstem Response (ABR) was measured up to 54 weeks post-injection and shows **significant permanent hearing recovery**. Graph represents median + SEM for all mice (one mouse was not corrected). **B,C.** P20-25 old *Otof*$^{KO/KO}$ mutant mice were injected with SENS-501 at two doses. WT and *Otof*$^{KO/KO}$ mutant mice injected with PBS were used as control. ABR for all groups was measured 10 months post-injection and shows **significant hearing improvement or restoration.** Both doses of SENS-501 demonstrated efficacy in improving hearing in KO mice, the high dose performing better at most of the frequencies. Restored <60dB; Improved <100dB on 3 consecutive frequencies. **D.** Overview of POC *in vivo* studies supporting the **long-term efficacy of SENS-501**.

5. Single intra-cochlear injection of SENS-501 in NHP: expression, 3-month GLP toxicity and biodistribution study & assessment of the hearing function

A

Group	Dose Level	Number of Animals	
		Males	Females
1	0 (vehicle)	3	3
2	Low Dose	3	3
3	High Dose	3	3



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Evaluation Results	End of Treatment
Systemic signs	**No SENS-501-related systemic signs observed**
Body Weight	**Not affected**
ECG	**Not affected**
FOB	**Not affected**
Body temperature	**Not affected**
Ophthalmology	**Not affected**
Injection site	**Not affected**
Clinical pathology, CRP	**No SENS-501 related changes**
Macroscopic/organ	**No SENS-501-related macroscopic or organ weights changes**
Systemic microscopic	**No SENS-501-related systemic microscopic findings, including DRG**
Local	**No SENS-501-related findings in the inner ear and vestibular system**
Hearing function	**No significant change vs placebo and vs baseline (ABR, DPOAE)**
Procedure-related microscopic	**No SENS-501-related findings**
Biodistribution	**Vectors mainly remained in injected ear structure**
Shedding	**SENS-501 quantification decreased over time down to non-detectable at Day 16 for all samples except ear swabs (3 months) at very low level**

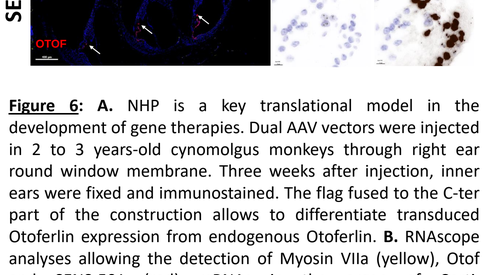

Figure 6: A. NHP is a key translational model in the development of gene therapies. Dual AAV vectors were injected in 2 to 3 years-old cynomolgus monkeys through right ear round window membrane. Three weeks after injection, inner ears were fixed and immunostained. The flag fused to the C-ter part of the construction allows to differentiate transduced Otoferlin expression from endogenous Otoferlin. **B.** RNAscope analyses allowing the detection of Myosin VIIa (yellow), Otof and SENS-501 (red) mRNAs in the organ of Corti, preponderantly in inner hair cells (IHC, arrows). SENS-501 injected ears are shown. Non-injected contra-lateral ears were also analyzed and confirmed the specificity of the staining observed in the injected ears (data not shown). **C.** Immunofluorescence staining of Otoferlin-flag show **specific expression of Otoferlin protein in inner hair cells** in injected ear (left) vs. no labeling in the contralateral ear (right). **D.** 3-Month GLP toxicity and biodistribution study in juvenile NHP. *In vivo* observations and pathology reporting at terminal euthanasia on Day 92: **SENS-501 was well tolerated and did not induce any macroscopic/organ weight changes or local/systemic microscopic findings at the end of the 3-month observation period.** Hearing function (ABR, DPOAE) was not affected by SENS-501. Biodistribution: no widespread distribution, no dissemination observed in gonads. Shedding: Vectors persistence in fluids was limited to blood (Day 4), urine (Day 16), feces (Day 16), nasal swabs (Day 16), saliva (Day 16), tears (Day 16) and ear swabs (3 months) at very low level.

Note: A 6-month SENS-501 GLP toxicity and biodistribution study in mice after IV injection confirmed good safety profile.

6. Program status

CRITERIA	SENS-501
Selective expression in target IHC cells	✓
Long-term efficacy data in preclinical mouse model	✓
Limited off-target tissue biodistribution	✓
Surgical approach developed and mastered by ENT surgeons	✓
Injection system validated for clinical use	✓
No correlation anti-AAV immunity and transduction efficacy	✓
GLP Tox – mice & NHP - No findings	✓
Drug Product manufactured under GMP conditions	✓
Clinical Trial Application approved in France	✓
OTOCONEX Natural History Study in Europe preparing execution of the clinical trial	→

Audiogene

Phase 1/2 Audiogene Study (SENS-501) Approved in France

First Patient Communication Anticipated in H2 2024

Conclusion

- SENS-501 administration results in target cell-restricted expression of Otoferlin in IHC in mice and NHP and long-term hearing restoration in mice.
- Dose-response experiments, early biodistribution studies in mice and NHP completed with limited off-target tissues exposure and no observed side-effect helped to design the GLP toxicity and biodistribution studies.
- SENS-501 was well tolerated and did not induce any macroscopic/organ weight changes or local/systemic microscopic findings in a 3-month GLP toxicity and biodistribution study conducted in NHP and 6-month in mice.

→ **Altogether, our nonclinical pharmacology, safety, biodistribution, and two ongoing natural history studies support the clinical development of SENS-501 and the initiation of our phase 1/2 clinical trial first half of 2024.**

→ **Phase 1/2 Audiogene Study (SENS-501) is approved in France. First Patient Communication Anticipated in H2 2024.**