Preclinical development of an Adeno Associated Vector-Based Gene Therapy (SENS-501) for the Autosomal Recessive Non-Syndromic Deafness 9 (DFNB9)

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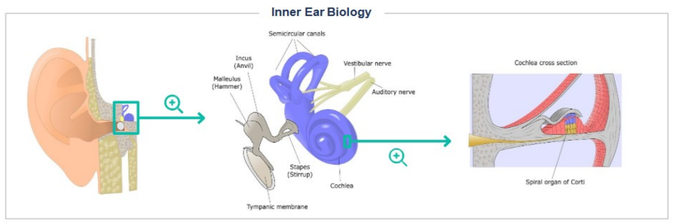
Abstract

Background: Autosomal recessive genetic forms (DFNB) account for most cases of profound congenital deafness. We focus here on the *Otoferlin* gene underlying DFNB9, one of the most frequent genetic forms of congenital deafness. Otoferlin is a calcium sensor protein critical for the transmission of the signal from inner hair cells (IHC) to the spiral ganglion neurons. DFNB9 is caused by pathogenic biallelic loss of function variations in *OTOF* gene leading to the failure of synaptic transmission, resulting in congenital severe-to-profound auditory neuropathy. Cochlear implantation is the only option proposed to young patients thus far. Although this medical device improves the quality of life and language acquisition, hearing quality is limited, and a treatment for DFNB9 is necessary to address this unmet medical need.

Method: We have developed SENS-501 as a dual AAV (Adeno Associated Virus) approach using two different recombinant vectors, one containing the 5' and the other the 3' portions of *Otoferlin* cDNA. SENS-501 was delivered into congenitally deaf DFNB9 mutant mouse inner ears through the round window at different doses. The therapeutic candidate, and a FLAG-tagged surrogate, was validated through demonstration of *Otoferlin* expression and integrity upon reconstitution of the full-length sequence in vitro and in vivo both in mice and NHP using immunohistochemistry. Dose-response experiments, early biodistribution studies after intracochlear injection were performed in mice and non-human primates (NHP). In primates, the surgical method and the delivery device were the ones envisioned in human. Biodistribution studies were conducted by qPCR and RT-qPCR. The stable, long-term, reversal of the deafness phenotype in *Otof*$^{-/-}$ mutant mice was evaluated through the assessment of auditory brainstem response (ABR) recordings after intra-cochlear administration either pre-hearing onset (immature cochlea) or in the mature cochlea and behavioral test allowing to demonstrate efficient auditory processing.

Results: IHC-restricted *Otoferlin* expression and good tolerability were demonstrated in both mice and NHP. Post-natal intracochlear injection of SENS-501 into the DFNB9 mutant mouse inner ear led to improvement of hearing thresholds and behavioral response as early as 3 weeks post-injection. Long-term ABR recovery was achieved in a dose dependent manner, with efficacy demonstrated for at least ten months. Dose-range, early biodistribution, as well as preliminary safety studies including immunogenicity with SENS-501 helped to design the ongoing GLP toxicity and biodistribution studies.

Conclusions: SENS-501 is safe and well tolerated in the GLP toxicity conducted in NHP. The selected AAV vector components allow to efficiently target IHC at levels compatible with therapeutic intervention in human and provide long-term efficacy data in DFNB9 mutant mouse model, which constitute a major step toward our future clinical trials to restore physiological hearing in DFNB9 patients.



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

1. Schematic hybrid dual AAV vector strategy (SENS-501)

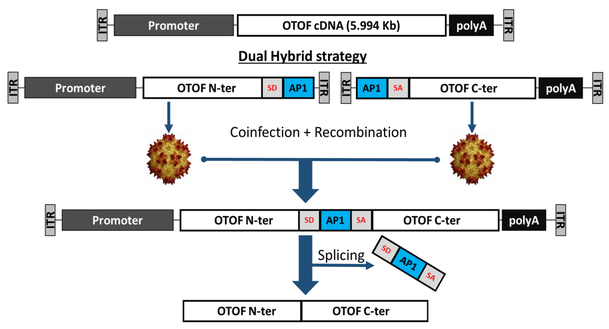


Figure 1: The size of the *OTOF* coding sequence largely exceeds AAV packaging capacity. To overcome this limitation, a dual AAV vector was designed, with *OTOF* coding sequence being split into two parts: i) a 5'-N-terminal (N-ter) vector, containing the N-terminal part of the AAV cassette, and ii) 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

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*

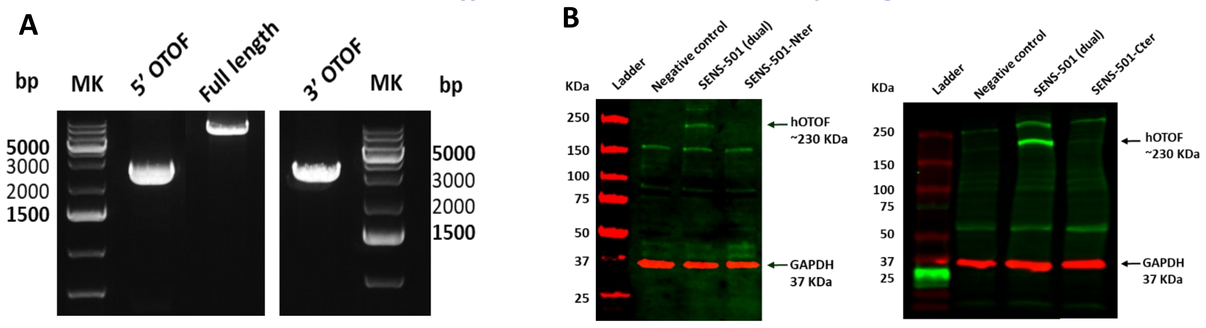


Figure 2: HEK-293 cells were coinfected with dual AAV vectors containing *Otoferlin* N-TER and C-TER sequences respectively (ratio 1:1).
A. RT-PCR on cells lysates show **highly efficient DNA recombination with full length mRNA amplification** and **B. highly efficient splicing with full-length Otoferlin 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*

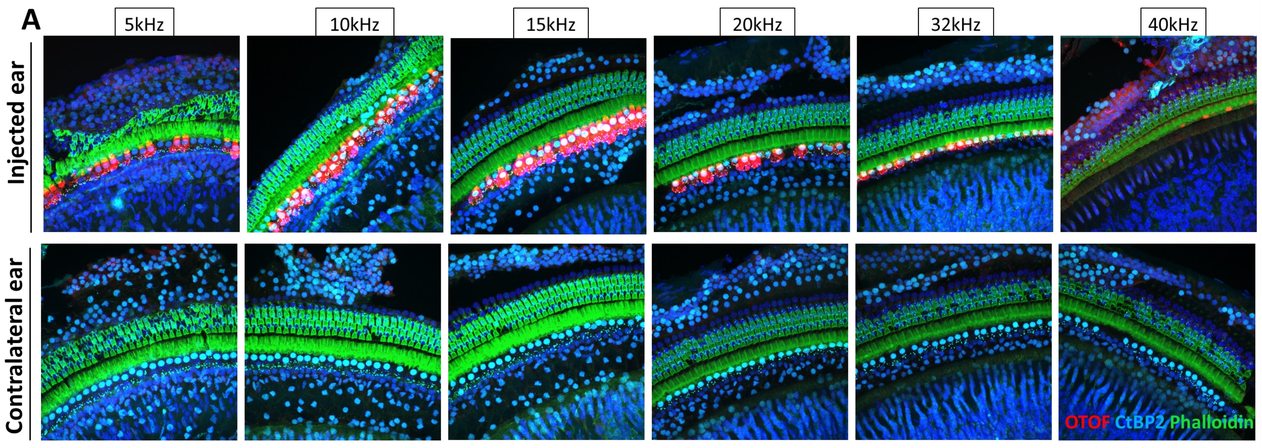


Figure 3: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing otoferlin transgene cassette. 6 months after injection, cochlea were sampled and stained for Otoferlin. **A. Otoferlin is *de novo* expressed in inner hair cells in injected left ear** (A-top) *vs.* no expression in 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 after injection of SENS-501*

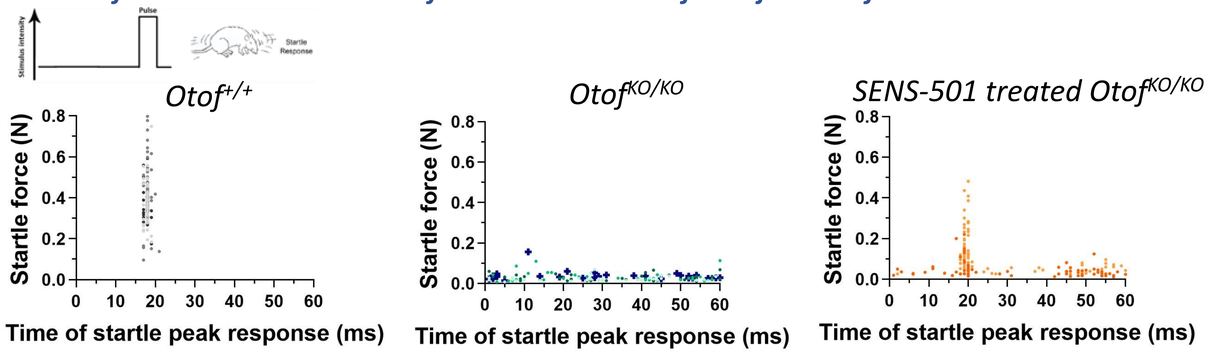


Figure 4: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing otoferlin transgene cassette. Schematic explanation of the acoustic startle reflex (ASR) assay (adapted from Turner & Parrish, 2008 and Kraus KS *et al.)* is shown. Startle reflexes are shown for wild type mice (Black)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

- *SENS-501 Leads to Long-term Hearing Recovery in a Translational Model of Otoferlin Deficiency*

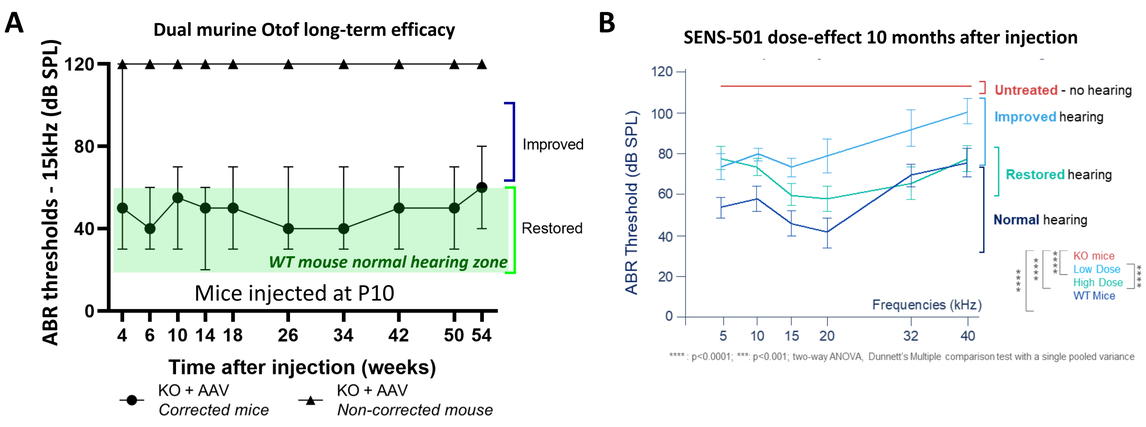
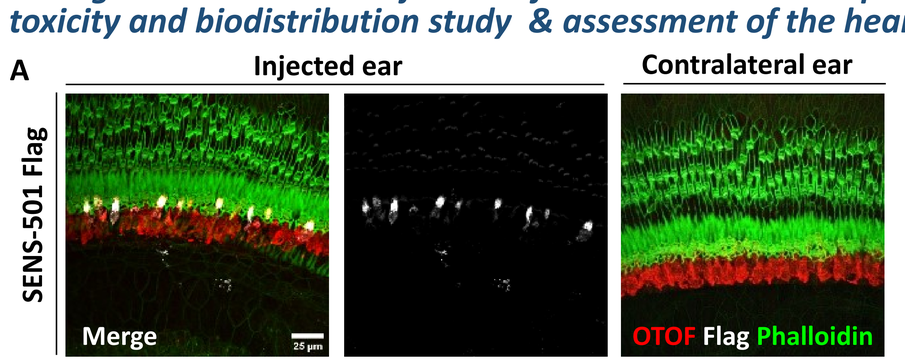


Figure 5: A. P10 old *Otof*$^{KO/KO}$ congenitally deaf mutant mice were injected with a dual vector containing the murine *otoferlin* transgene cassette (n=8). Auditory Brainstem Response (ABR) was measured up to 54 weeks post-injection and shows **significant permanent hearing recovery**. Graph represents 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**.

4. Single Intra-Cochlear Injection of SENS-501 in NHP: expression, 3-month GLP toxicity and biodistribution study & assessment of the hearing function



B

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

C

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**

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. Immunofluorescence staining of Otoferlin-flag show s**pecific expression of Otoferlin protein in inner hair cells** in injected ear (left) vs. no labeling in the contralateral ear (right). **B,** 3-Month GLP toxicity and biodistribution study design. Juvenile NHP at injection were used
C. 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.

5. 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	✓
No findings – early safety /tolerability studies – mice & NHP	✓
No correlation anti-AAV immunity and transduction efficacy	✓
GLP Tox studies under completion – mice & NHP	→
Natural History Study preparing execution of the clinical trial	→
Regular engagement with regulatory agencies	✓
Drug Product manufactured under GMP conditions	✓

Clinical Trial Application planned H1 2023

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. In NHP, we achieved an effective transduction rate of the targeted IHC at levels compatible with therapeutic intervention**, which constitutes a **major step toward future clinical trials in DFNB9 patients**.
- **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.**
- **Sensorion is pursuing on track to file a Clinical Trial Application for the program in H1 2023.**