SA82. 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: *OTOF* is a gene expressed in the inner hair cells (IHC) of the cochlea and encodes for Otoferlin. Otoferlin is a calcium sensor protein critical for the transmission of the signal from IHC to the spiral ganglion neurons (SGNs). The autosomal recessive deafness 9 (DFNB9) is caused by pathogenic biallelic loss of function variations in *OTOF* 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. **Methods:** We have developed SENS-501, a dual hybrid Adeno-Associated Virus (AAV) vector for DNFB9. Indeed, the size of the *OTOF* coding sequence largely exceeding AAV packaging capacity, *OTOF* coding sequence has been split into two AAV vectors. SENS-501 was delivered into congenitally deaf DFNB9 mutant mouse ears through the round window (RW) at different doses. In parallel, SENS-501 was administered to Non-Human Primates (NHP) using the surgery and device that will be used in human. Early tolerability and biodistribution of SENS-501 studies were conducted in both species. **Results:** The therapeutic candidate 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. In both species, IHC-restricted Otoferlin expression and good preliminary tolerability were demonstrated. Post-natal intracochlear injection of SENS-501 into the DFNB9 mutant mouse inner ear led to improvement of hearing thresholds as early as 3 weeks post-injection, and long-term auditory-evoked brainstem responses, in a dose dependent manner, with efficacy demonstrated for at least six months. Dose-response experiments, early biodistribution studies in mice and NHP after intracochlear injection were performed. **Conclusions:** SENS-501 appears safe and well tolerated. 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 future clinical trials in DFNB9 patients. Dose range finding studies and early biodistribution studies, as well as the good preliminary safety profile of SENS-501, helped to design the ongoing GLP toxicity and biodistribution stun studies.

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

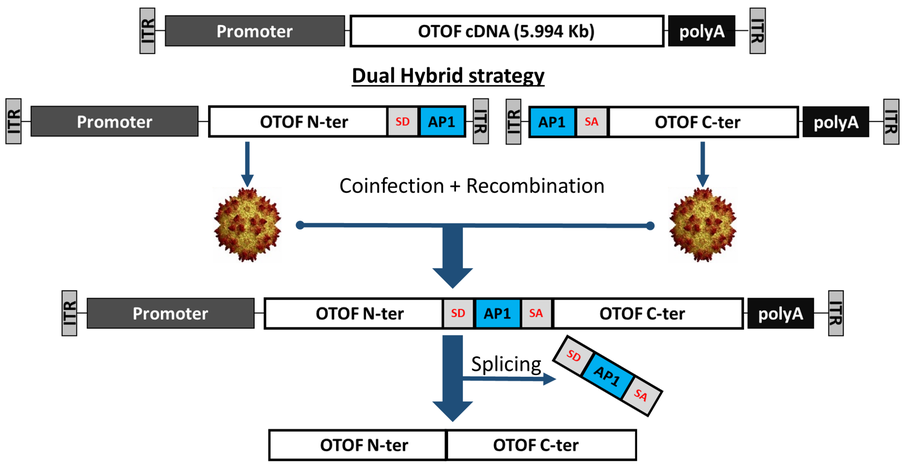


Figure 1: The size of the *OTOF* coding sequence largely exceeds AAV packaging capacity. To overcome this limitation, a dual AAV vector was designed, with *OTOF* coding sequence being split into two parts: i) a 5'-N-terminal (N-ter) vector, containing the N-terminal part of the AAV cassette, and ii)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

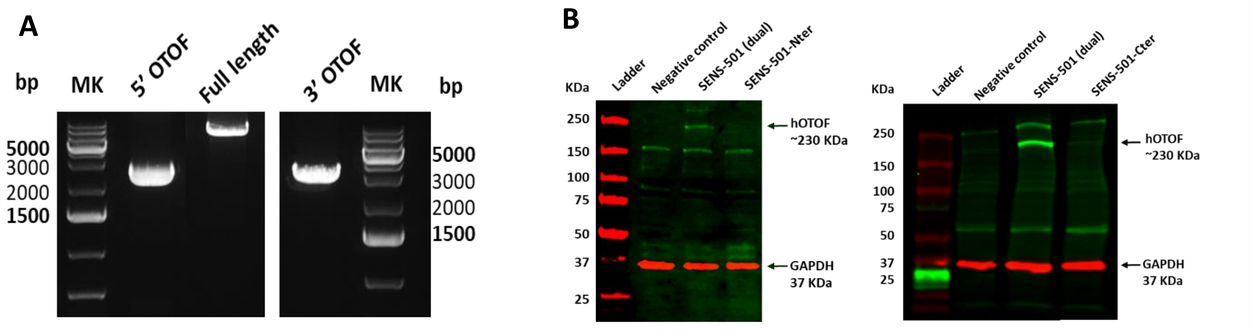


Figure 2: HEK-293 cells were coinfected with dual AAV vectors containing *Otoferlin* N-TER and C-TER sequences respectively (ratio 1:1).
A. RT-PCR on cells lysates show **highly efficient DNA recombination with full length mRNA amplification** and **B. highly efficient splicing with full-length Otoferlin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

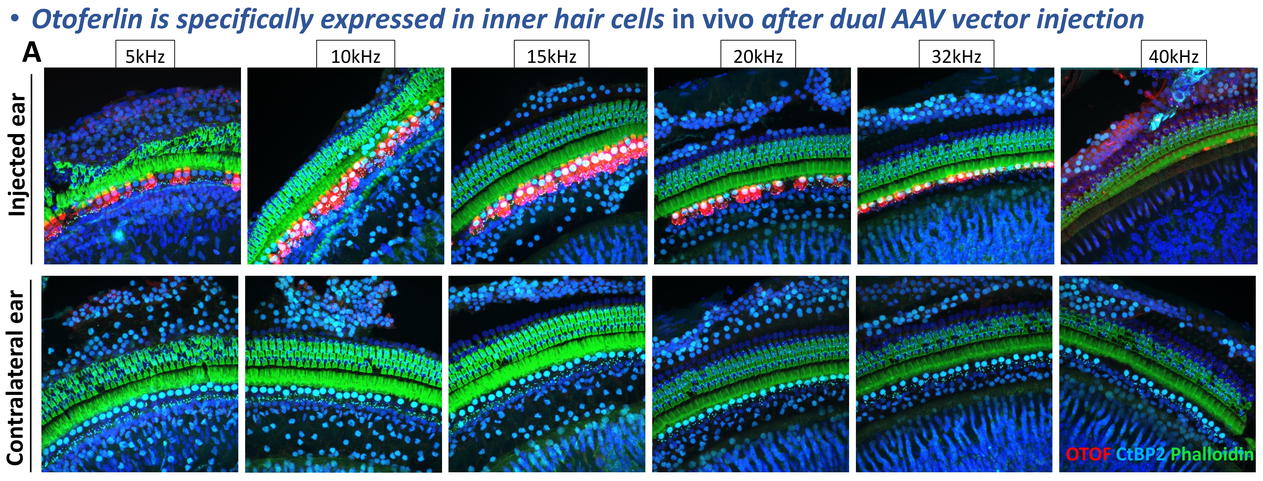


Figure 3: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing otoferlin transgene cassette. 6 months after injection, cochlea were sampled and stained for Otoferlin. **A. Otoferlin is *de novo* expressed in inner hair cells in injected left ear** (A-top) *vs.* no expression in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

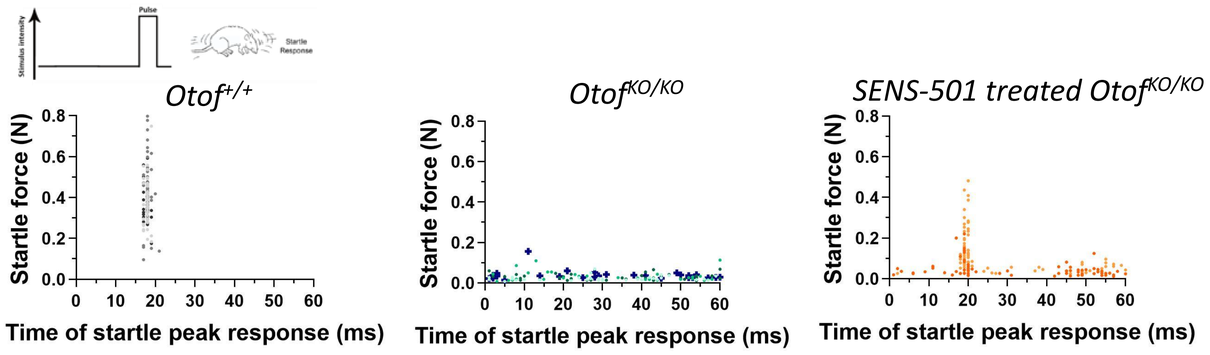


Figure 4: *Otof*$^{KO/KO}$ mutant mice were injected with the dual vector containing otoferlin transgene cassette. Schematic explanation of the acoustic startle reflex (ASR) assay (adapted from Turner & Parrish, 2008 and Kraus KS *et al.)* is shown. Startle reflexes are shown for wild type mice (Black) 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.

- *ABR threshold measurements of Otof*$^{KO/KO}$ *mutant mice are improved up to 1 year after injection*

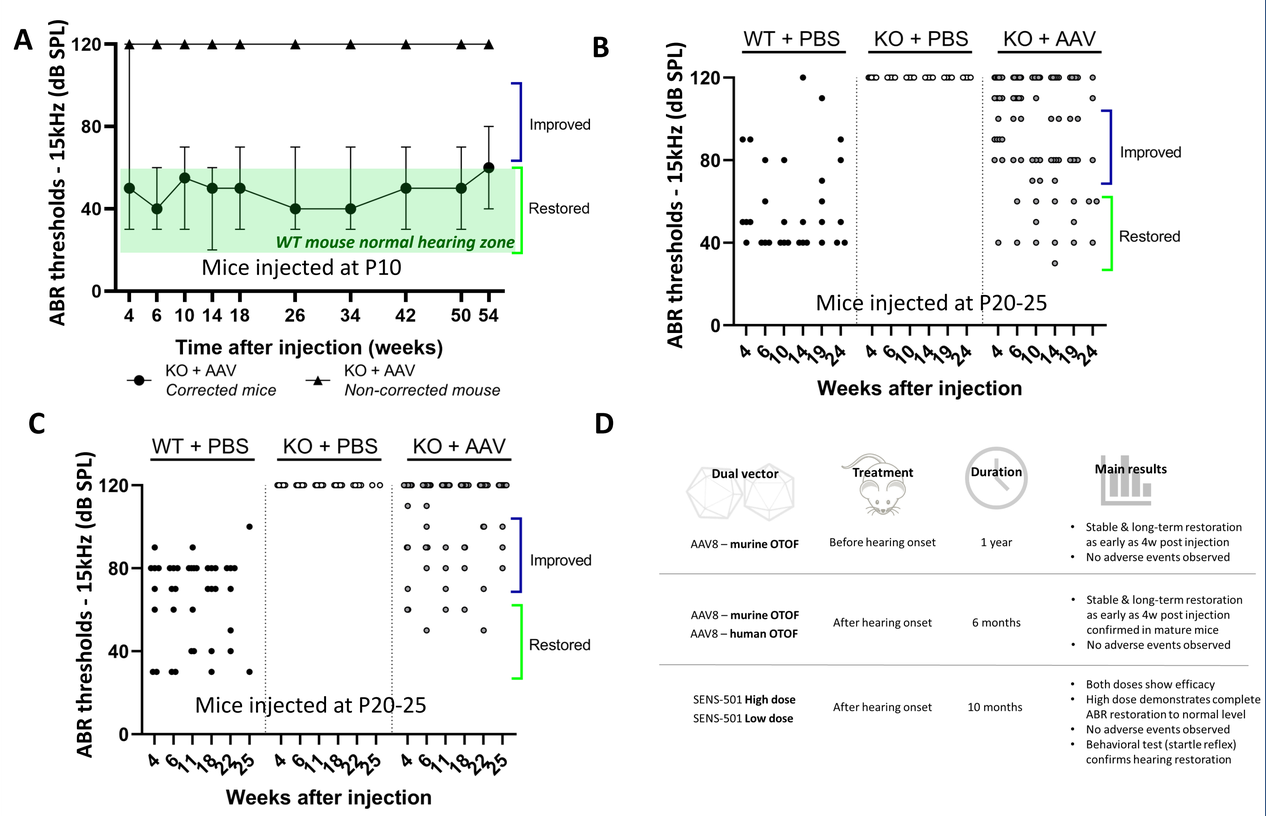


Figure 5: A. P10 old *Otof*$^{KO/KO}$ mutant mice were injected with a dual vector containing the murine *otoferlin* transgene cassette (n=8). Auditory Brainstem Response (ABR) was measured up to 54 weeks post-injection and shows **significant permanent hearing recovery**. Graph represents median + SEM for all mice (one mouse was not corrected). **B,C.** P20-25 old *Otof*$^{KO/KO}$ mutant mice were injected with a dual vector containing either the murine (B) or the human (C) *Otoferlin* transgene cassette. WT and *Otof*$^{KO/KO}$ mutant mice injected with PBS were used as control. ABR for all groups was measured up to 24+ weeks post-injection and shows **significant hearing improvement or restoration for both vectors**. Restored <60dB; Improved <100dB on 3 consecutive frequencies. **D.** Overview of POC *in vivo* studies supporting the efficacy of SENS-501.

4. SENS-501 biodistribution in NHP shows limited detection in other organs

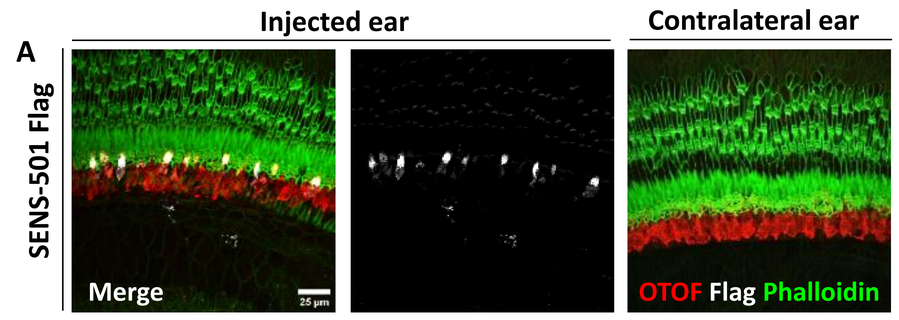
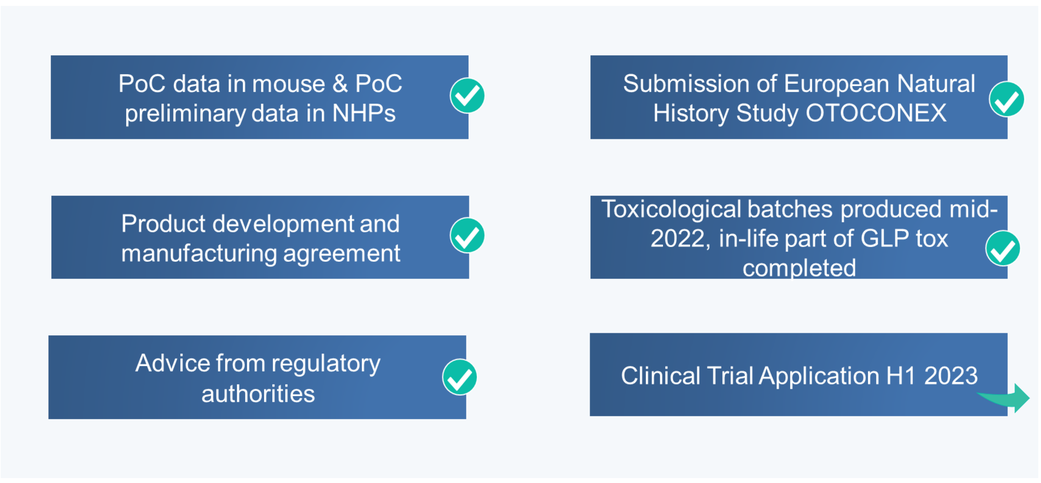


Figure 6: A. NHP is a key translational model in the development of gene therapies. Dual AAV vectors were injected in 2 to 3 years-old cynomolgus monkeys through right ear round window membrane. Three weeks after injection, inner ears were fixed and immunostained. The flag fused to the C-TER part of 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,C.** Organs of injected NHPs were collected for quantitative evaluation of AAV dual vectors biodistribution by qPCR (B) or hOTOF mRNA by RT-qPCR (C). NHP#1 to #7 were injected with AAV dual vector, NHP#8 and #9 were injected with vehicle. NHP#6 and #7 were injected with a higher dose of vector. We observed **limited AAV leakage restricted to CSF-bathed tissues with no mRNA expression in off-target tissue.**
GC: Genome Copy
Red dotted line: detection threshold

5. Program status



Conclusion

- We demonstrated a **target cell-restricted expression of Otoferlin in IHC in mice and NHP** and **long-term hearing restoration in mice after SENS-501 administration**. 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 **ongoing GLP toxicity and biodistribution studies.**
- **EMA and FDA recently issued ODD in 2H 2022 and the program is eligible for Rare Pediatric Voucher. Sensorion is pursuing on track to file a Clinical Trial Application for the program in H1 2023.**