575. Adeno-Associated Vector Discovery Platform For Inner Ear Disorders

Arnaud PJ. Giese [1], Patrice Vidal [1], Montserrat Bosch Grau [1], Audrey Broussy [1], Selma Dadak [1], Mickael Deage [1], Vincent Descossy [1], Guillaume Olivier [1], Mathieu Petremann [1], Christophe Tran Van Ba [1], Julie Duron Dos Reis [1], Pauline Liaudet [1], Laurent Désiré [1]

[1] Sensorion, Montpellier, France

Abstract

Around 466 million people worldwide have hearing loss. It is estimated that over 900 million people will have disabling hearing loss by 2050. More than half of the congenital non-syndromic deafness cases have a genetic cause, and 80% are inherited in an autosomal recessive fashion. There are no approved curative therapies for genetic hearing loss and cochlear implantation is the only option proposed to young patients. Even though this solution improves the quality of life and language acquisition, hearing recovery is limited, and thus more targeted treatments are overall unmet medical needs. Adeno-associated virus (AAV) is a vector of choice for in vivo gene therapy. Many serotypes, either natural or synthetic, allow using the vector for several indications. As the number of clinical trials and successes is increasing, the field is reaching bottlenecks including issues with immunogenicity, toxicity, vector quantity and expression specificity. The inner ear is a closed system which contains a small volume. The organ contains 3 major types of highly specialized functional cells: sensory cells (about 3,500 inner hair cells and 12,000 outer hair cells) but also supporting cells, and spiral ganglion neurons, all of which play an important role in the process of hearing and therefore could be selectively targeted by gene therapy. Therefore, the production of a gene therapy product, especially for the inner ear, requires the development of novel tissue-targeted capsids and cell-specific expression cassettes aiming to reach a therapeutic effect using a minimal dose. Here we describe the newly developed platform at Sensorion, allowing fast screening of AAV candidates for inner ear indications.

1. The Adeno-Associated Vector Discovery Platform at Sensorion.

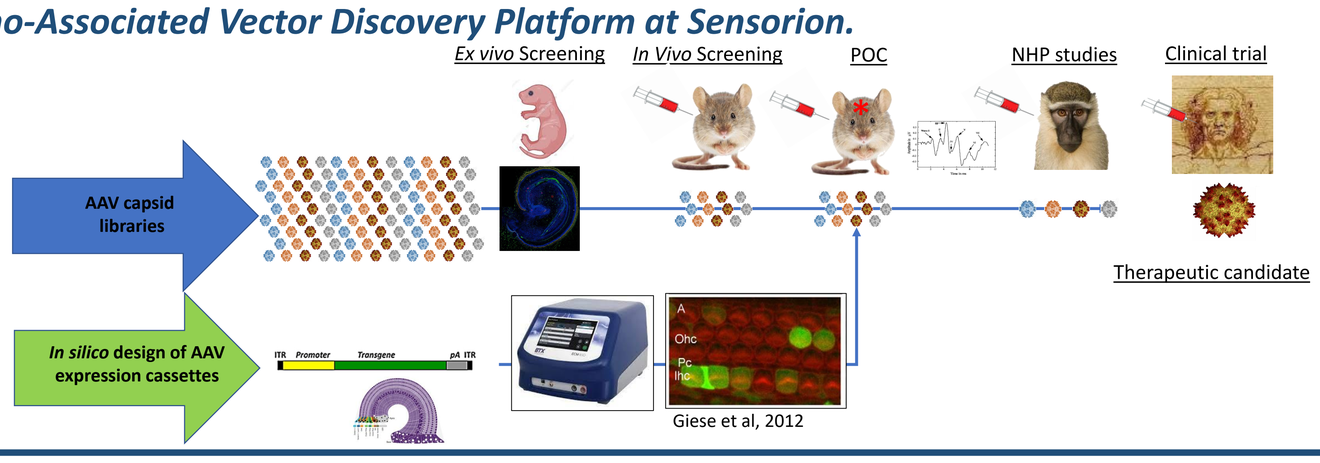
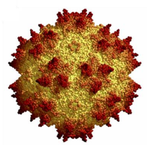


Figure 1: Flowchart of the high throughput selection process of Sensorion's Adeno-Associated Vector Discovery Platform that has been designed to sort the best capsids and regulatory element among tens of candidates in few weeks. The platform allows to culture mouse inner ear explants and infect them with AAV libraries. Selected candidates are further validated by in vivo injection into the inner ear using a canalostomy or round window injection techniques. Inner ear function, and ototoxicity are assessed via Sensorion's audiology platform which includes auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) recordings, and morphological and immunohistochemical examination.
AAV expression cassettes of interest are investigated by electroporation of inner ear explants. Candidate AAV expression cassettes are validated in mouse mutant models for proof of concept (POC). Therapeutics candidates are further validated in non-human primate (NHP) studies for transduction, efficiency, biodistribution and potential toxicity. New proprietary therapeutic products to treat inner ear disorders are then assessed in clinical trial.

2. AAV Capsid Screening performed ex vivo for the discovery of AAV vectors suitable for gene therapy in the inner ear

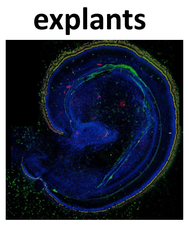
A. Work flow and capacity of our AAV capsid screening platform

AAV capsid libraries



- AAV-GFP
- Screening 20/week in duplicate + controls

Ex vivo explants



- Cochlea
- Vestibule
- Stria Vascularis

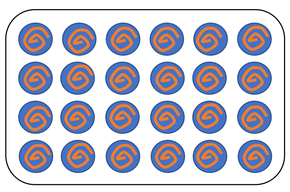
Immunostaining and Confocal imaging

- GFP amplification
- Co-immunostaining with inner ear markers
- High-content Confocal imaging

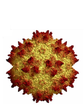
Figure 2: A. Workflow and capacity of our AAV capsid platform. The screen can be performed on vestibular, cochlear explants as well as primary culture of stria vascularis cells. The cultures are infected with a library of AAV-GFP vectors, co-immunostained with inner ear cell markers validated in house in combination with a GFP amplification. A High-content screening system, extremely sensitive confocal imaging and high throughput through simultaneous acquisition allows us to analyze infected explants on 24 well plates and assess the tropism and cell transduction efficiency of each capsid. **B.** Mouse cochlear explants are dissected at P2 and infected after in culture with a constant number of AAV-GFP. After several days, explants are fixed and co-immunostained with inner ear markers and GFP signal is amplified. Cochlear explants are imaged in a 24-well plate using the Opera Phenix system. Z-stacking, Tiles and stitching are performed, then the tropism and transduction efficiency of each capsid is assessed and quantified.

B. Our optimized protocol in six steps

1. Mouse cochlear explant Culture (Embryonic->P5)



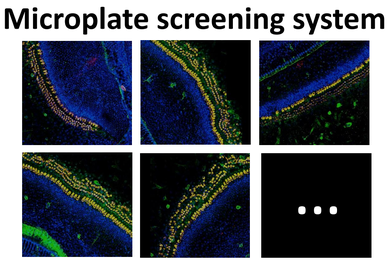
2. AAV Infection



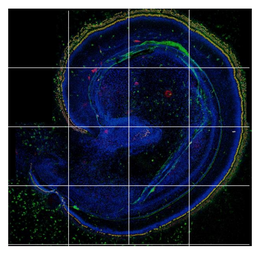
3. Co-Immunostaining (up to 4 channels)

GFP/MyoVIIa/Prox1/DAPI
GFP/NF200/MyoVIIa/DAPI
etc…

4. High-content Confocal Microplate screening system

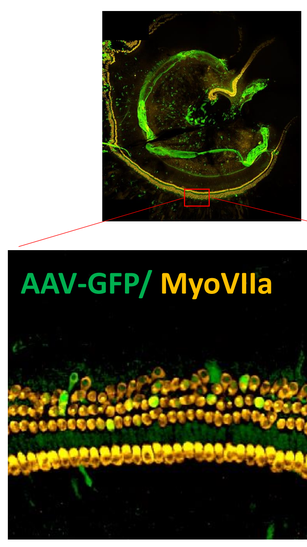


5. Z-stacking, Tiles and Stitching



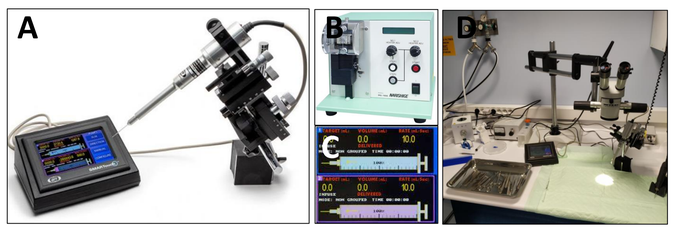
GFP/MyoVIIa/DAPI

6. Assessment of tropism and cell transduction efficiency

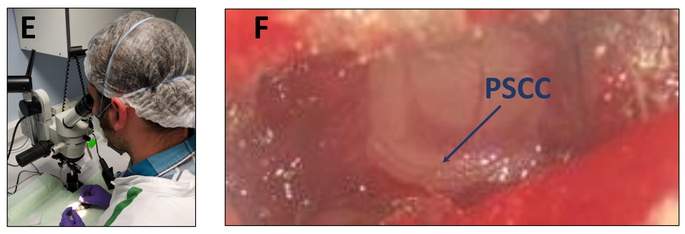


3. In Vivo Gene Therapy Rodent Platform

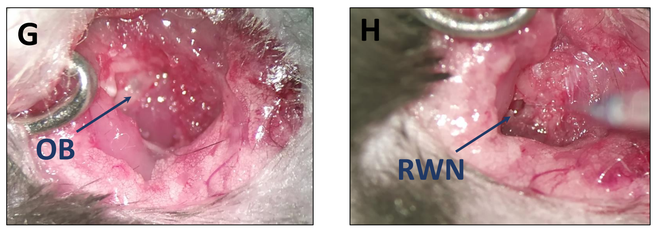
In Vivo microinjection apparatus



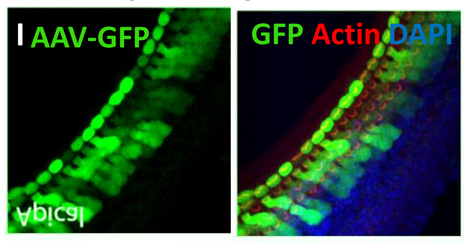
Canalostomy of the posterior semicircular canal



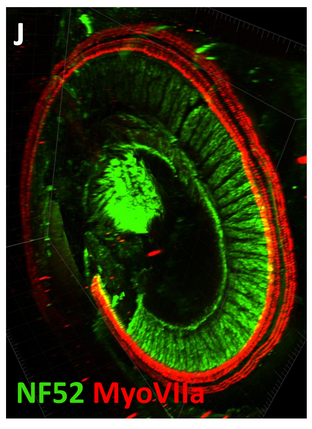
Round window membrane injection



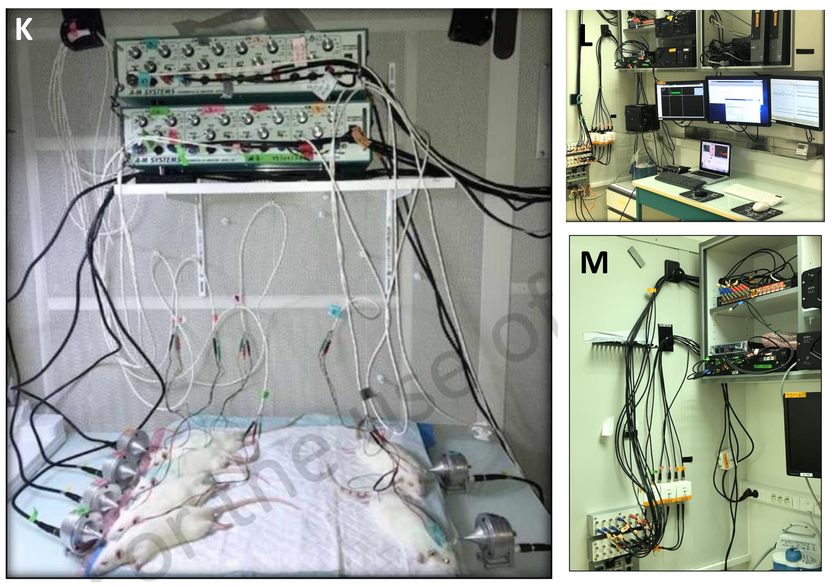
In Vivo injection of AAV into the mouse inner ear



3D Histological analysis of proteins localized in the mouse inner ear



Custom-built multichannel ABR and DPOAE platform for rats and mice



Pure-tone ABR and DPOAE measurements

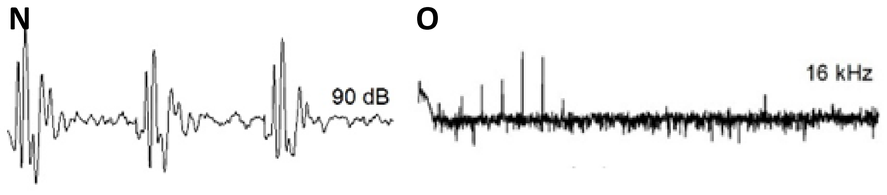


Figure 3: A. Nanoliter2020 Injector apparatus used for precise nanoliter-volume injections along with a with a SMARTouch™ controller. **B, C.** Nanoliter volume injections are performed using glass micropipettes. Borosilicate glass capillaries are pulled with the Narishige PC-100 designed specifically for pulling ø1mm~1.5mm O.D. pipettes. **D.** Round window and posterior semi-circular injections are performed under a binocular. **E.** Experimentator performing a canalostomy in an adult mouse is shown. F. The posterior semicircular canal (PSCC) of the vestibular system is shown. **G, H.** An injection of AAV into the round window membrane of the adult mouse inner ear is performed. OB: Otic bulla, RWN: round window niche. **I.** Histological analysis of the cochlea of a mouse that received an injection of AAV-GFP (green) into the posterior semicircular canal at P0. Stereociliary bundles are counterstained with phalloidin (red). **J.** Tissue clearing and imaging of an inner ear immunostained with MyoVIIa (hair cell) and NF52 (neurofilament) markers. **K, L, M.** Our custom-built auditory brainstem responses (ABR) workstation and sound proof chamber are shown. The workstation allows us to assess the hearing function of 8 animals simultaneously. The TDT RZ6 Auditory Workstation is used to perform DPOAE measurements **N,O.** Auditory Brainstem Responses (tone-pips of 2 msec, 20/s, at 8/16/24 kHz, band-pass filtered from 0.1-5 kHz, custom-built ABR workstation) and Distortion Product Otoacoustic Emissions (f1 and f2 = 70 dB SPL, f2/f1 ratio of 1.2, at 4/8/16/24/32 kHz, TDT RZ6 Auditory Workstation) were recorded in closed-field configuration with animals deeply anesthetized using ketamine/xylazine/acepromazine.

Conclusion

- In vitro high throughput selection process allowing to sort the best capsids and regulatory element among tens of candidates in few weeks.
- The platform allows to culture rodent cochlear, vestibular and stria vascularis explants and cultures, and either infect them with AAVs or electroporate them with plasmids of interest.
- The imaging acquisition has been fully automatized for efficient data analysis and quantification.
- Selected candidates are further validated by in vivo injection into the inner ear using a canalostomy or round window injection approach.
- Inner ear functions are assessed via Sensorion's audiology platform which includes auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) recordings.
- The relevant tests are assessed in large groups of animals and are supported by morphological and immunohistochemical examination in rodents and NHPs.
- The gene therapy platform allows Sensorion to identify and validate novel AAV variants and cell-type specific expression cassettes of interest for new proprietary therapeutic products to treat inner ear disorders.