Assessment of Next-Generation AAV Variants in Gerbil and Non-Human Primate Retina Following Intravitreal Injection

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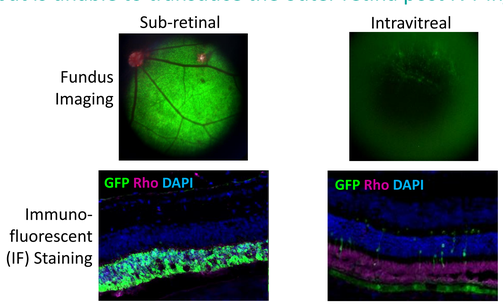
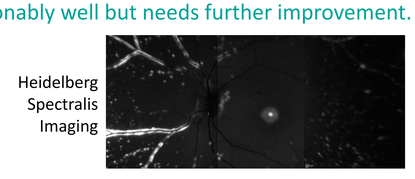
Background

- AAV-mediated gene therapy using sub-retinal (SR) injections is promising for some ocular diseases.
- Intravitreal (IVT) injection of current AAV serotypes, while less invasive, is far less efficient due to the inner limiting membrane (ILM), which is a physical barrier to these vectors in the primate retina.
- In order for AAV vectors to cross the ILM adequately, they need to bind heparan sulfate proteoglycan (HSPG) receptors to a certain degree (Dalkara et al, 2009).
- R585 and R588 are the two primary residues on AAV2 that are involved in HSPG binding (Opie et al, 2003).
- 2.5T is a chimeric variant of the VP1 region of AAV2 and VP2 and VP3 regions of AAV5 containing a single A581T point mutation (Excoffon et al, 2009).
- It binds to sialic acid receptors and has no affinity for HSPG.
- 2.5T efficiently transduces photoreceptors when injected SR, but is unable to transduce the outer retina post IVT injection.



- ShH10 was discovered by *in vitro* directed evolution and is a variant of AAV6 with 3 point mutations (Klimczak et al, 2009).
- ShH10 transduces the non-human primate (NHP) retina reasonably well but needs further improvement.



- 2.5T and ShH10 have better neutralizing antibody (nAb) profiles compared to AAV2.

Purpose

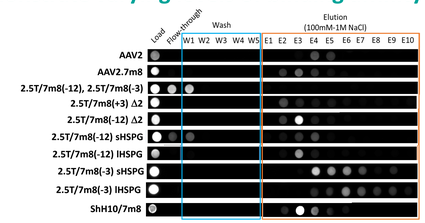
- We are developing novel AAV vectors for clinical indications with IVT delivery that can overcome current limitations – cross the ILM and have a favorable nAb profile.
- 2.5T and ShH10 have certain characteristics that are advantageous for an ocular gene therapy vector – they can transduce retinal cells and have an immune profile that is better than AAV2.
- However, 2.5T is unable to transduce the outer retina after IVT injection on account of its inability to bind HSPG receptors that are abundant on the ILM.
- For effective IVT transduction, we attempted to alter the binding affinity of 2.5T/7m8 hybrid variants to HSPG receptors to an optimal degree – not too strong or too weak, by swapping in the HSPG-binding residues from AAV2.
- Also, we sought to improve retinal tropism of ShH10 after IVT delivery by inserting a 10 amino acid peptide in its receptor-binding surface-exposed region.

Methods

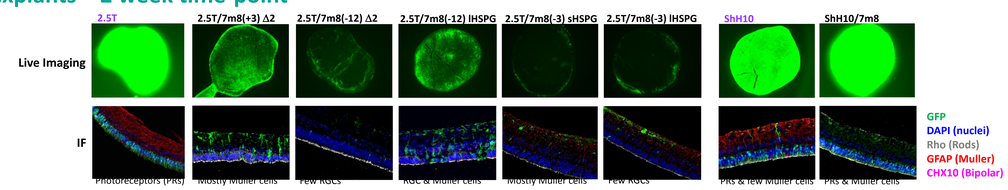
- After 3D structural comparison, site-directed mutagenesis was performed to generate several 2.5T/7m8-HSPG variants.
- Similarly, after 3D structural assessment of AAV6 (parent capsid for ShH10), the 10-aa 7m8 loop was inserted into the receptor binding region of ShH10.
- These variants, carrying the GFP marker gene were produced in HEK293 cells using a standard protocol and were characterized by qPCR to establish titer and packaging, western blot to ensure correct ratio of the VP1, VP2, and VP3 capsid proteins.
- For HSPG binding assay, a HiTrap™ Heparin HP column (1mL) was used. 2E11 vg were loaded, then washed, and finally eluted with increasing concentrations of NaCl (100mM – 1M). Fractions of load, flow-through, wash, & elution were collected and then analyzed on a dot-blot using the α-AAV B1 antibody.
- *Ex vivo* pig retinal explants were transduced at 2E10 vg. Live fluorescent images were captured followed by IF staining at 2 week.
- *In vivo* studies were performed in gerbils and AGMs where variants were IVT injected at 2E10 vg or 2.5E12 vg per eye, respectively. Fluorescence fundus images were captured at various times followed by IF staining of retina at termination.

Results & Conclusion

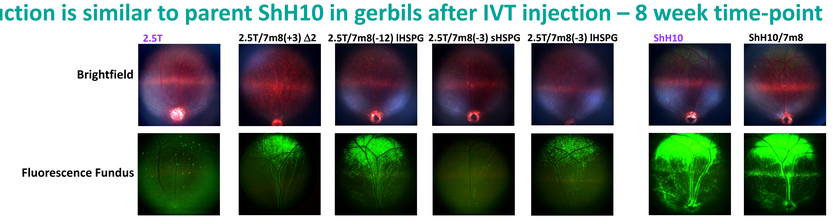
- **Characterization**
  - A majority of variants packaged efficiently, resulting in titers greater than E12 vg/ml.
  - Western blot showed the correct ratio of VP1, VP2, and VP3 capsid proteins.
- **2.5T/7m8-HSPG variants demonstrate varying levels of binding affinity to heparin**



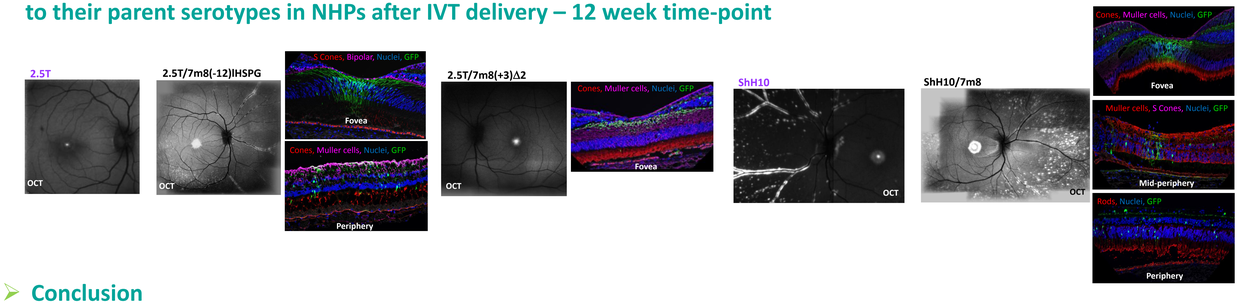
- **2.5T/7m8-HSPG and ShH10/7m8 variants display variable levels of transduction and altered tropism to retinal cells in pig explants – 2 week time-point**



- **Some of the 2.5T/7m8-HSPG variants exhibit enhanced transduction compared to parent 2.5T. However, ShH10/7m8 transduction is similar to parent ShH10 in gerbils after IVT injection – 8 week time-point**



- **2.5T/7m8(-12)IHSPG and ShH10/7m8 demonstrate superior transduction, noted by higher GFP expression compared to their parent serotypes in NHPs after IVT delivery – 12 week time-point**



➢ **Conclusion**

Inserting either the HSPG binding site or a loop in the receptor binding regions of 2.5T/7m8 and ShH10 respectively, did indeed improve transduction of the retina following intravitreal delivery.

Disclosures

AK, MN, CC, and MG are employed by Avalanche Biotechnologies Inc.

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