Nonclinical Safety Assessment of SPVN06, an AAV-based Gene Therapy for the Treatment of Rod-Cone Dystrophies

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Introduction

Rod-Cone dystrophies (RCD) are inherited retinal diseases characterized by progressive loss of the rod photoreceptors, followed by cone photoreceptor degeneration, eventually leading to total blindness. 71 genes are identified in RCD, mostly affecting the rods. **SPVN06 aims at slowing down the degeneration of cones** by restoring RdCVF trophic support normally provided by rods, and by promoting RdCVFL antioxidant activity. RdCVF and RdCVFL are encoded in the same AAV-based vector. SPVN06 single subretinal administration is expected to slow down cone degeneration in RCD patients independently of the causative mutation. **SPVN06 nonclinical safety was evaluated in non-human primate (NHP, cynomolgus monkeys) for 3 months.**

Materials & Methods

3 males and 3 females per group received a bilateral subretinal administration at dose levels ranging from 6.0E+9 to 3.0E+11 vg/eye. Animals were followed up to 3 months with interim euthanasia at 1-mo for 6.0E+10 and 3.0E+11 vg/eye doses. All animals received an immunosuppressive regimen administration starting the day prior dosing and then weekly for 4 weeks (Methylprednisolone, 40 mg/animal, intramuscular (IM)) to mitigate any potential immunologic reaction to AAV and/or to the transgenes. A standard toxicological assessment was conducted including an exhaustive battery of ocular testing (gross examination, slit lamp, ocular fundus examination, intraocular pressure, full-field electroretinography (ffERG), optical coherence tomography (OCT)). Histopathology analysis was conducted on an extensive list of tissues and immunohistochemistry (IHC) staining were performed on eye paraffin sections. Immunogenicity assessments included total antibodies against AAV and RdCVF/L as well as T-cell mediated toxicity (ELISpot). SPVN06 DNA and RdCVF/L mRNA expression were respectively quantified using qPCR and RTqPCR in the retina and exhaustive list of tissues.

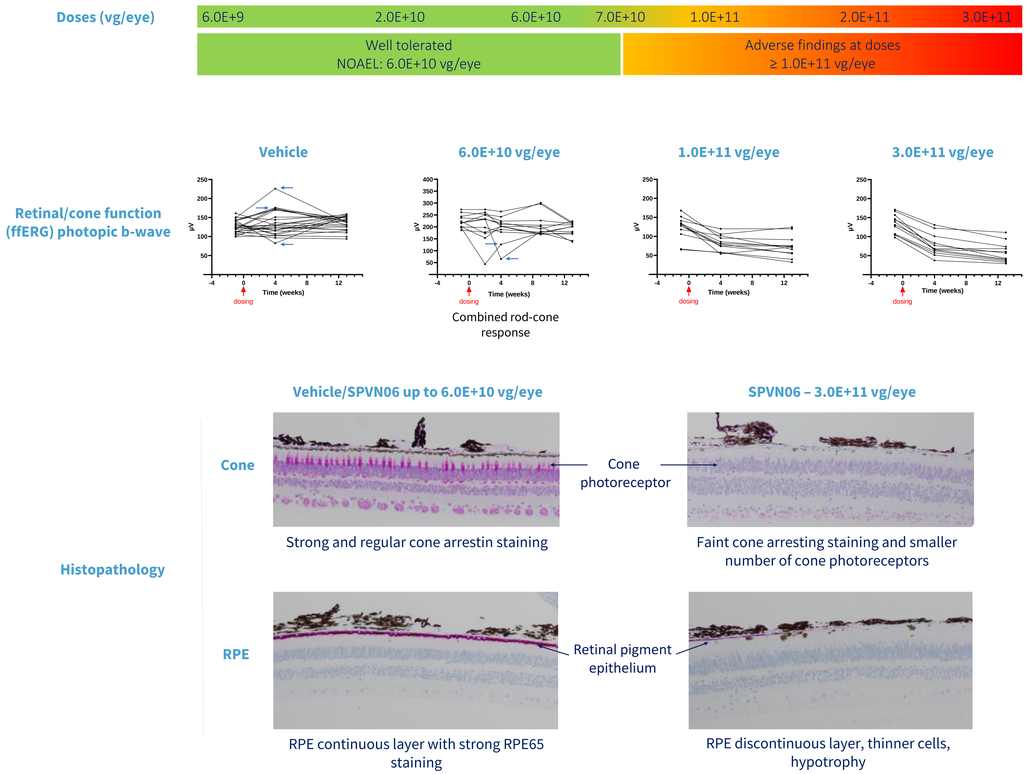
Results

There were no drug-related systemic effects. SPVN06-related adverse findings were noted at ≥1.0E+11 vg/eye and were limited to the photoreceptors and retinal pigment epithelium (RPE) cells. These findings were dose-related and characterized by reduced ffERG amplitude with microscopic correlates. In absence of an immune response to the transgenes and consistently with their mechanism of action, this toxicity was deemed attributed to the supraphysiological levels of transgenes in a healthy retina. The role of AAV to the observed toxicity couldn't be completely ruled out but was unlikely a major contributor considering that the uveitis was transient and the presence of immune infiltrate minimum.

Study design

Treatment	Dose level (vg/eye)	Volume injection (µL/eye)
Vehicle	0	100
	0	150
SPVN06	6.0E+9	100
	2.0E+10	100
	6.0E+10	100
	7.0E+10	100
	1.0E+11	100
	2.0E+11	100
	3.0E+11	150

SPVN06 safety profile in NHPs

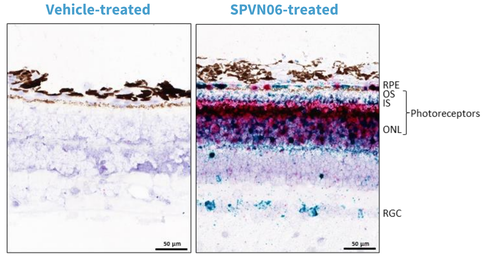


Ocular observations: transient injection-related ocular inflammation was observed in all animals (vehicle and SPVN06 treated-animals) after dosing that peaked at D7/D14 and gradually resolved by 3 months (data not shown).

Retinal function: No major ffERG changes were observed in the vehicle nor in SPVN06 groups up to 6.0E+10 vg/eye, except minor changes at 1-month (blue arrows) that correlate with the transient injection-related ocular inflammation. SPVN06-related adverse findings were notice at doses ≥1.0E+11 vg/eye and were dose-related and characterized by reduced ffERG amplitude (rod and cone responses) with microscopic correlates. Partial ffERG recovery was observed at 1.0E+11 vg/eye.

Histopathology: Cone photoreceptor and retinal pigment epithelium (RPE) specific staining were also performed (cone arrestin and RPE65 respectively). Analysis of the retina after H&E revelated no adverse findings in vehicle and SPVN06-treated animals up to 6.0E+10 vg/eye. SPVN06-related adverse findings at doses ≥1.0E+11 vg/eye were observed and mainly limited to the photoreceptors and RPE cells. Mild to marked decreases in cone arrestin staining was observed in the peripheral and central retina of most animals that indicated loss of the cone cell population. These observations correlated with the retina functional decline as evidenced by reductions in the ffERG a- and b-wave amplitudes. All animals also exhibited minimal to moderate decreases in RPE65 staining of the peripheral RPE. A IHC rod-specific staining was not conducted. In addition, transient immune cell infiltrates in photoreceptor layer were detected in interim necropsy at 1-month at 3.0E+11 vg/eye.

Biodistribution in NHP retina

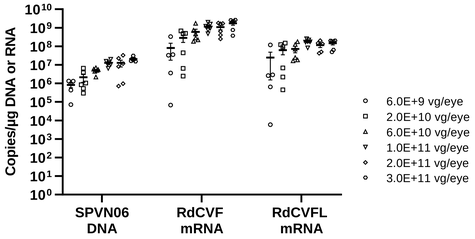


RPE: retinal pigmented epithelium; OS: outer segments; IS: inner segments; ONL: outer nuclear layer; RGC: retinal ganglion cells

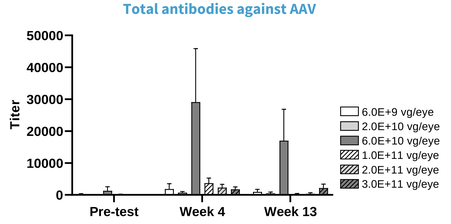
Confirmation of SPVN06 cell transduction and transgene expression *in vivo at* 7.0E+10 vg/eye, with high expression of both transgenes in the photoreceptor layer and RPE at 1-month post-injection.

SPVN06 DNA and transgenes mRNA expression were quantified in NHP retina bleb 3 months after dosing. A dose response is observed with the increased doses of SPVN06. 1-mo interim data at 6.0E+10 and 3.0E+11 vg/eye demonstrated roughly steady levels of SPVN06 DNA and transgenes mRNA between 1 and 3 months after dosing (data not shown).

mean ± SEM, n=6 except for RdCVFL mRNA at 2.0E+11vg/eye where n=5 and for all 6.0E+11 analysis where n=5 .



Immune response: limited to transient anti-AAV antibody response



As expected, transient and non-adverse immune response against the AAV capsid (total antibodies) was observed whereas no immune response against the transgenes (total antibodies) was detected. Indeed, most animals had pre-existing anti-AAV total antibodies, that peaked 4 weeks after SPVN06 administration and then decreased at week 13. There was no obvious relationship between titer and doses. No T-cell response either against capsid or transgenes was detected (ELISpot). Mean ± SEM, n=6 except for 2.0E+11 vg/eye dose n=4.

Conclusion

No SPVN06-related adverse findings were noted up to 6.0E+10 vg/eye, the NOAEL was thus determined at 6.0E+10 vg/eye.

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