Nonclinical safety and pharmacokinetic assessment of SPVN06, an AAV-based gene therapy for the treatment of rod-cone dystrophies

Poster ID
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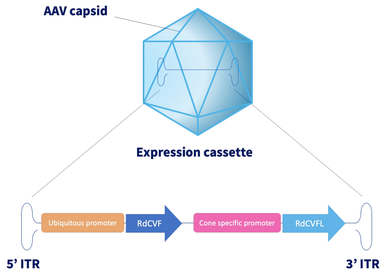
Purpose

Rod-cone dystrophies (RCD) are inherited retinal diseases characterized by photoreceptor degeneration, eventually causing blindness. In all RCD, degeneration is first observed in rods, and subsequently in cones, in large part due to a lack of trophic support. More than 1.5 million individuals worldwide are affected by RCD with numerous genes identified.

The *NXNL1* gene encodes two proteins, rod-derived cone viability factor (**RdCVF**) and its full-length isoform, thioredoxin **RdCVFL**, produced by rods and rods/cones respectively. These trophic factors support cone survival by promoting glycolysis and preventing oxidative damage, respectively.



SPVN06 is an adeno-associated viral vector encoding the short and long forms of RdCVF/L cDNAs under the control of a ubiquitous and cone specific promoters, respectively.

SPVN06 is currently being tested in patients with Retinitis Pigmentosa, the most common form of RCD, of various genetic etiologies for its tolerability and its potential to promote cone photoreceptor survival upon subretinal administration, independently of the causative mutation.

Methods

SPVN06 safety evaluation was conducted in non-human primates (NHP). Three studies were performed in cynomolgus monkeys as components of combination endpoint studies that included pharmacokinetics (PK) and toxicology evaluations. These studies, including two 3-month GLP studies evaluated SPVN06 absorption, biodistribution and shedding, as well as transgenes mRNA expression. These studies evaluated dose levels of SPVN06 ranging from 6E9 to 3E11 vg/eye administered via a single bilateral subretinal injection.

Biodistribution was determined by qPCR method that detects SPVN06 vector genome DNA, and RT-qPCR that detects *RdCVF* and *RdCVFL* mRNA. Absorption and shedding were determined by measuring the vector copy number by qPCR in whole blood and tears, respectively.

Primers and probes were designed to specifically target the exogenous *RdCVF* and *RdCVFL* mRNAs produced following SPVN06 delivery to distinguish them from endogenous mRNAs. SPVN06 vector genome and *RdCVF* and *RdCVFL* mRNA levels are reported as absolute quantifications, per µg of DNA or RNA, respectively.

Additionally, a RT-PCR method was developed for the investigation of the broad distribution profile of RdCVFL.

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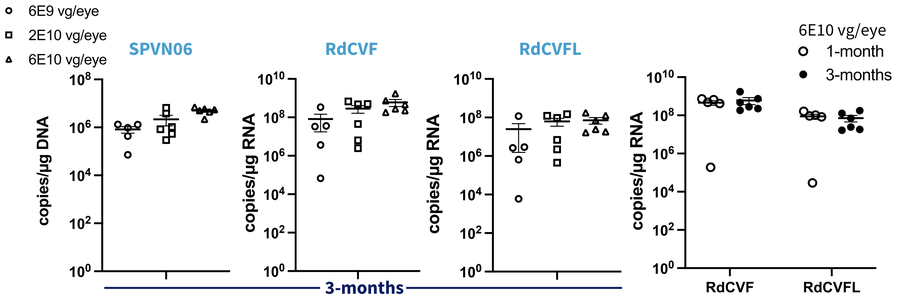
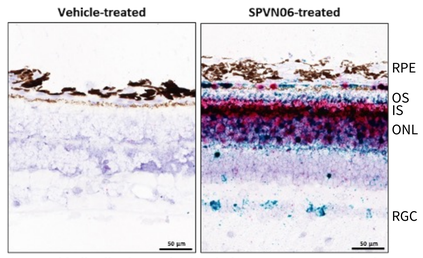
Disclosures - SparingVision employees: ASG, MME, LC, HK, FL / SparingVision personal interest: MME, TL, HK, JAS, FL/ Patents: TL, JAS / Received financial support and consultant: GP, TL, JAS

Results

➢ Biodistribution

Retinal tissues





SPVN06 biodistribution and *RdCVF*/*RdCVFL* mRNA expression in NHP retina (*In situ* Hybridization)
Photoreceptors: OS (Outer Segments)+IS (Inner Segments)+ONL (Outer Nuclear Layer); RPE: Retinal Pigment Epithelium; RGC: Retinal Ganglion Cell
Target probes: Green for SPVN06 vector genome, Red for *RdCVF* and *RdCVFL* mRNA

SPVN06 vector genome
- High and sustained levels up to 3 months in the retina
- Significant distribution outside the bleb
- Limited detection in non-ocular tissues

***RdCVF* and *RdCVFL* mRNA**
- High and sustained levels in the retina
- Significant distribution outside the bleb
- Limited expression in non-ocular tissues
- Broad expression of *RdCVFL* mRNA in ocular tissues despite the cone specific promoter

Ocular and non-ocular tissues

Copies/µg DNA

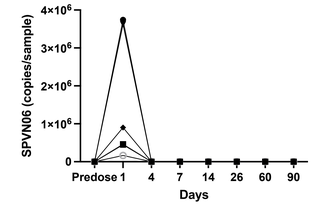
Biodistribution profile of SPVN06 1-mo post dosing in NHP (vg/eye)

	6E10 vg/eye (n=6)	3E11 vg/eye (n=4)
Retina Left Bleb	7.9×10^{6}	2.2×10^{7}
Retina Left Remaining	1.3×10^{6}	4.9×10^{6}
Choroid/RPE bleb	8.8×10^{6}	2.5×10^{7}
Choroid/RPE remaining	1.8×10^{6}	6.1×10^{6}
Sclera remaining/Sclera bleb	3.9×10^{5}	1.3×10^{6}
Iris/ciliary body	1.7×10^{5}	3.2×10^{5}
Lens	4.1×10^{4}	1.2×10^{5}
Bulbar and palpebral conjunctiva	1.2×10^{3}	3.7×10^{5}
Cornea	1.4×10^{4}	7.7×10^{5}
Optic nerve	4.5×10^{4}	3.7×10^{4}
Aqueous humor	0	1.0×10^{1}
Vitreous humor	1.1×10^{2}	6.8×10^{2}
Mandibular lymph nodes	3.3×10^{4}	6.0×10^{4}
Optic chiasm	1.1×10^{4}	1.5×10^{5}
Optic tract	4.0×10^{3}	1.3×10^{4}
Spleen	2.2×10^{4}	5.6×10^{4}
LGN	7.7×10^{2}	5.2×10^{3}
Liver caudate	1.2×10^{2}	2.3×10^{3}
Liver lateral left	1.2×10^{2}	2.0×10^{3}
Liver lateral right	1.4×10^{2}	1.8×10^{3}
Liver median	1.5×10^{2}	1.9×10^{3}
Lung	1.3×10^{2}	3.0×10^{1}
Superior colliculus	3.9×10^{1}	5.5×10^{1}
Visual cortex	0	0
Frontal cortex	0	0
Cerebellum	0	0
Kidney	2.0×10^{1}	0
Heart	0	6.8×10^{1}
Ovary	0	0
Testis	0	0

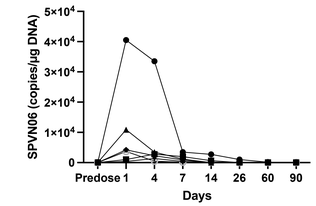
0 = samples are either BLD (Below Limit of Detection) or BLQ (Below Limit of Quantification).

➢ Shedding

Tears (e.g. OS)

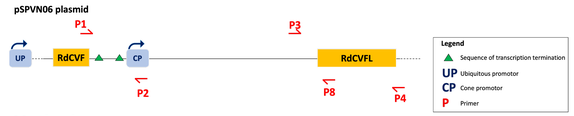


Blood



SPVN06 vector genome (6E10 vg/eye)
Transiently detected in tears and blood

➢ Broad expression of *RdCVFL* RNA : long transcripts hypothesis



Method
- Transduction of WERI-Rb-1 human retinoblastoma cells (MOI 3E5 vg/cell) and RNA extraction
- 4 assays selected for RT-PCR analysis: P1-P2, P3-P4, P1-P8 and P1-P4

Results
- No specific amplification for any of the RNA samples analyzed
- No long transcripts including RdCVF sequence and spanning beyond the transcription termination sequence were observed

➢ **Broad expression profile could be related to transcriptional interference due to the influence of a strong and ubiquitous promoter on a weaker tissue-specific promoter**

➢ Safety profile summary

6E9 | 6E10 | 1E11 | 3E11 | Vg/eye

Well Tolerated NOAEL is 6E10 vg/eye	**Adverse findings at ≥1E11 vg/eye ↓ ffERG ↓Photoreceptor layer and RPE**
AAV Tab – transient increase	
Procedure-related transient ocular inflammation was noted in both vehicle and SPVN06-treated eyes	
No anti-transgene antibodies No T-cell mediated toxicity against AAV or transgenes (ELISpot)	
No systemic findings	

➢ Phase I/II clinical trial

- **NHP Safety Study** is the driver for setting the clinical dose levels
  - ➢ Relationship between **Dose, Distribution and Safety**
  - ➢ Dose scaling is based on retinal surface

Reasonable safety data supporting the full clinical dose range

NOAEL in NHP	Human equivalent dose	Clinical dose level	Dose-multiple margin
6E10 vg/eye	1.2E11 vg/eye	6E9 vg/eye	20-fold
		2E10 vg/eye	6-fold
		6E10 vg/eye	~2-fold

Retinal surface

- ***rd10* mouse pharmacology data (data not shown)**
  - ➢ Relationship between **Dose and Efficacy**
  - ➢ Dose scaling is based on transgenes expression in the retina

Efficacy is expected at the lower clinical dose

Highly Active dose in mice	NHP equivalent dose	Human equivalent dose
1E8 vg/eye	5E9 vg/eye	1E10 vg/eye

Transgenes mRNA expression | Retinal surface

Conclusions

Biodistribution - shedding

✓ Transient presence of SPVN06 in blood and tears

✓ High tropism for the retina and RPE/Choroid cells

✓ Sustained expression levels of the transgenes, up to 3 months

Safety

✓ SPVN06-related changes limited to ocular findings at dose levels ≥ 1E11 vg/eye

✓ Safety findings were limited to the photoreceptors and RPE, and were attributed to overexpression of transgenes in a healthy monkey retina and/or to overload of vector particles

✓ No anti-transgene antibodies detected, and no T-cell mediated toxicity against the AAV capsid or the transgenes

→ **NOAEL: 6E10 vg/eye**

First-in-Human Clinical trial PRODYGY: SPVN06-CLIN-01 NCT05748873; EUCT05748873