Pre-clinical toxicology of AAV2/5-OPTIRPE65, an optimised *RPE65* gene therapy vector

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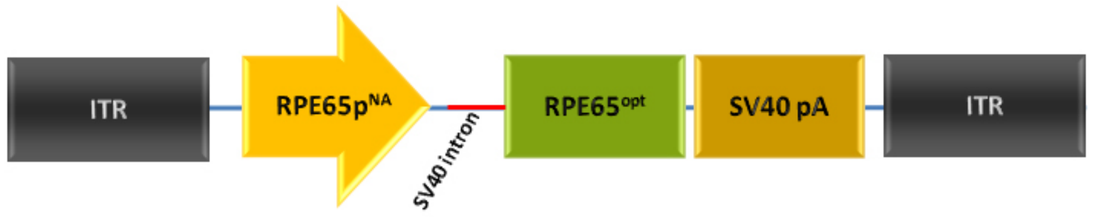
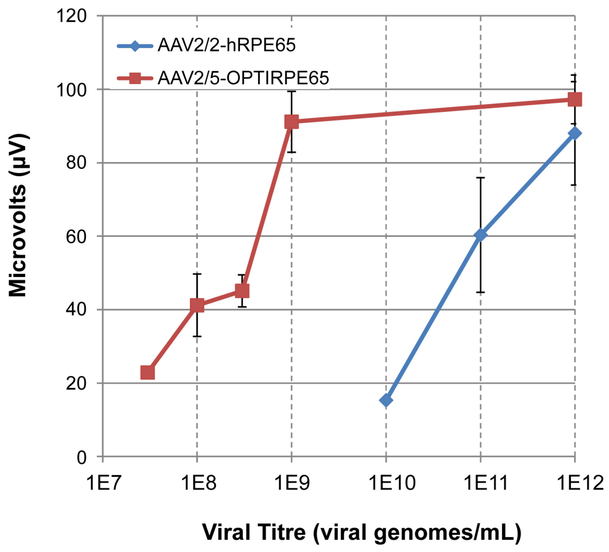
Introduction

Leber congenital amaurosis (LCA2), a childhood retinal dystrophy caused by mutations in the *RPE65* gene has been the focus of various gene therapy clinical trials. Most trials showed modest improvements in (night) vision that were generally not sustained, most likely due to an insufficient level of *RPE65* expression in the human RPE. We developed AAV2/5-OPTIRPE65, an AAV2/5-based vector, carrying an optimised expression cassette comprising a stronger promoter, intron and codon-optimised transgene (1).





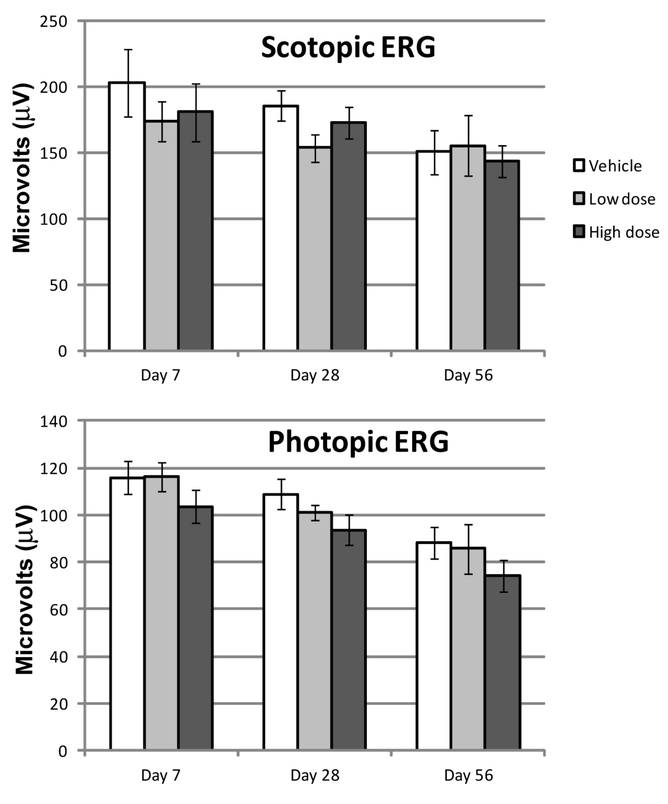
A head-to-head comparison between AAV2/5-OPTIRPE65 and the AAV2/2-based gene therapy vector used in our first Phase I/II clinical trial measuring retinal sensitivity of Rpe65-deficient mice following subretinal delivery of either vector at increasing titres was performed. Maximum attainable scotopic responses (~90µV) were achieved with a 300-fold lower titre of AAV2/5-OPTIRPE65.

Toxicology – Retinal function & structure

In preparation for a Phase I/IIa clinical trial, the safety of GMP grade AAV2/5-OPTIRPE65 was assessed in wild-type mice (C57Bl/6J), Rpe65-deficient mice ($Rpe65^{rd12/rd12}$) and wild-type rabbits (New Zealand White). Retinal function and structure were assessed to determine whether ocular toxicity was associated with high levels of RPE65 protein in the wild-type.

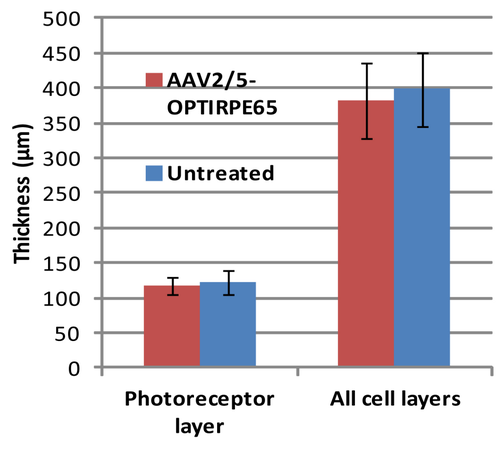
Wild-type mice and rabbits were assessed in an acute toxicological study probing for toxicity at 7, 28 and 56 days. Mice were also used in a longer study with a 9 month endpoint. The acute toxicological studies assessed the effect of a Low vector dose ($1.3x10^9$ vg/eye in mice, $0.6x10^{11}$ vg/eye in rabbits), a High vector dose ($4x10^9$ vg/eye in mice, $2x10^{11}$ vg/eye in rabbits) and Vehicle. The long-term study assessed a High dose ($4x10^9$ vg/eye).

In all the studies and all assessed time-points there were no significant decreases in scotopic or photopic electroretinography (ERG) responses.
Retinal thickness measurements were obtained from all eyes after the end of in-life phase and no significant decreases were observed in either the photoreceptor cell layer or the thickness of the whole retina.



Rod-mediated (scotopic) and cone-mediate (photopic) ERG responses in wild-type mice at 7, 28 and 56 days after subretinal delivery.

Retinal thickness measurements of high dose treated versus untreated retinas in wild-type mice at 9 months after subretinal delivery



Biodistribution – Vector dissemination

Real-time PCR for vector genome sequences was quantified in absolute numbers against a standard curve of amplicon DNA.

In both mice and rabbits major organs were sampled for the analyses (see table below for major tissues in the mouse studies / High dose results) with gonads and tissues of the contralateral uninjected eye included in the rabbit studies.

Some dissemination of vector was observed for both species leading to detection of vector genomes mainly in the liver, as it would be expected. Low level dissemination in lymph nodes and adrenals was also detected.

Detection was more prominent in early time-points (i.e. 7 days after administration).

Vector Copy number/µg of DNA

High dose	Adrenals	Brain	Heart	Kidney	Liver	Lungs	Lymph nodes	Spleen
Day 7 – 11	290	-	BDL	BDL	16400	BDL	140	BDL
Day 7 – 12	120	BDL	BDL	BDL	2060	BDL	4760	BDL
Day 7 – 13	1220	BDL	BDL	BDL	10200	BDL	1880	BDL
Day 7 – 14	110	BDL	BDL	BDL	10500	BDL	BDL	BDL
Day 7 – 15	4230	BDL	BDL	BDL	11400	BDL	BDL	BDL
Day 28 – 26	230	-	-	-	350	-	7420	-
Day 28 – 27	160	-	-	-	BDL	-	740	-
Day 28 – 28	150	-	-	-	200	-	250	-
Day 28 – 29	240	-	-	-	250	-	220	-
Day 28 – 30	310	-	-	-	330	-	BDL	-
Day 56 – 41	BDL	-	-	-	BDL	-	BDL	-
Day 56 – 42	BDL	-	-	-	BDL	-	BDL	-
Day 56 – 43	BDL	-	-	-	BDL	-	BDL	-
Day 56 – 44	BDL	-	-	-	BDL	-	BDL	-
Day 56 – 45	BDL	-	-	-	BDL	-	BDL	-

BDL; below the limit of detection

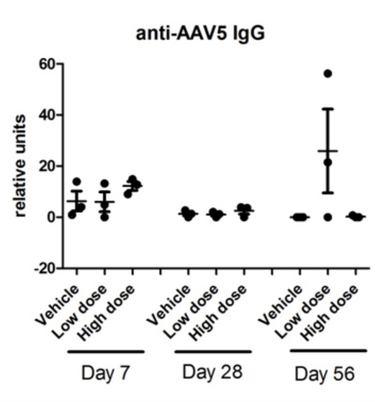
Anti-AAV5 immune responses

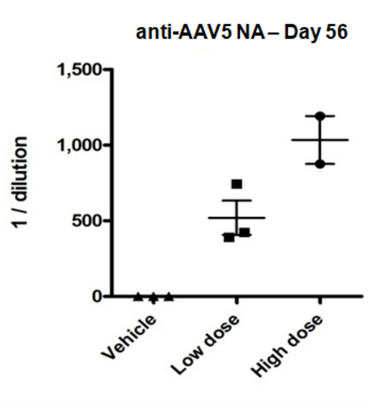
Anti-AAV5 capsid antibodies and anti-AAV5 neutralising antibody (NA) titres were established. Data for the rabbit acute toxicological study are presented.

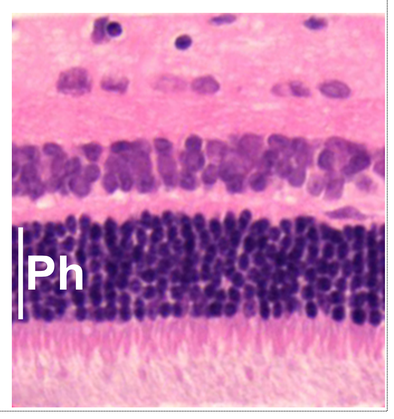
Anti-AAV5 IgG responses were seen in one mouse and one rabbit injected with the Low dose at the 28 day time-point. No increase in anti-AAV5 IgG was detected in any of the mice or rabbits receiving High dose AAV2/5-OPTIRPE65.

Neutralising antibodies against the AAV5 capsid were consistently found in vector injected animals after Day 7, as expected. Previously, subretinal administration of AAV2/2-hRPE65 gave rise to neutralising antibody titres up to 1 in 1000 in trial participants without safety concerns (2).

Histological analysis of all animals did not reveal signs of retinal disorganisation. A representative image of a treated rabbit retina is shown below with the photoreceptor layer indicated (Ph).







Conclusions

Administration of AAV2/5.OPTIRPE65 did not adversely affect retinal structure or function. Low level vector dissemination mainly to the liver was detected without pathological changes in these tissues. As expected, we detected some immune responses against the vector capsid, without concomitant pathology.
We conclude that subretinal administration of AAV2/5-OPTIRPE65 is safe for use in clinical trials.

References:
1. Georgiadis *et al.*, Gene Therapy (2016), 23: 857-862.
2. Bainbridge *et al.*, N Engl J Med (2015) 14;372(20): 1887-1897.

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