

I concur with this review. M. Serabian 11/16/17

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PRODUCT:                                      LUXTURNA™ (voretigene neparvovec; AAV2-hRPE65v2: adeno-associated viral vector serotype 2, expressing normal human retinal pigment epithelial 65 kilodalton protein gene)

APPLICANT:                                   Spark Therapeutics

PROPOSED INDICATION:                LUXTURNA™ is a gene therapy product indicated for treatment of patients with vision loss due to confirmed biallelic RPE65 mutation-associated retinal dystrophy.

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**EXECUTIVE SUMMARY:**

*In vitro* pharmacology studies performed using normal retinal pigmented epithelial (RPE) and RPE65 mutant canine cells and human HEK293T cells transduced at various multiplicities of infection (MOIs) of AAV2-hRPE65v2 showed dose-dependent expression of the RPE65 protein.

*In vivo* pharmacology studies were conducted in *RPE65*<sup>-/-</sup> mice and *rd12* mice. Subretinal injection of AAV2-hRPE65v2 (1 x 10<sup>9</sup> vg/mouse eye) resulted in: 1) expression of the RPE65

protein only in the RPE cells in the injected eye in both models, 2) improvement in electroretinography (ERG) in both models, and 3) improvement in visual acuity as measured by optokinetic responses, for the *rd12* mice.

Subretinal delivery (bilateral, simultaneous administration) of AAV2-hRPE65v2 vector ( $8.25 \times 10^{10}$  vg/dog eye) into RPE65 mutant dogs resulted in improved pupillary responses, ERGs, and visual behavior, and reduced nystagmus. RPE65 protein was detected in the RPE cells located in the portion of the retina exposed to the vector. Subretinal delivery (bilateral, sequential, with one eye injected at day 0 and the contralateral eye injected 13-15 days later) of AAV2-hRPE65v2 ( $1.5 \times 10^{11}$  vg/dog eye) resulted in improved navigation and pupillary responses within two weeks post-first eye injection. Further improvement in visual behavior and diminution in nystagmus was observed post-second eye injection.

Single dose and repeat dose toxicology studies were conducted in normal-sighted dogs, RPE65 mutant dogs, and normal-sighted non-human primates (NHPs). Dosing regimens for the single dose studies consisted of either unilateral administration or bilateral, simultaneous administration. Several subretinal dosing regimens were employed for the repeat dose studies. The clinically relevant schedule consisted of injection of vector product into one eye, followed (at an interval of at least 2 weeks) by injection into the contralateral eye (bilateral, sequential administration). Additional dosing regimens included: 1) administration to one eye, followed (at an interval of 98 days) by administration to the contralateral eye, followed by re-administration to one of the eyes (bilateral, sequential/re-administration) and 2) administration to both eyes, followed (at an interval of approximately 30 days) by re-administration to one of these eyes (bilateral, simultaneous/re-administration; ipsilateral).

Following single or repeat subretinal administration of AAV2-hRPE65v2 in dogs and NHPs, no toxicity was observed in non-ocular tissues. Occasional inflammation in the retina, attributed to the surgical delivery procedure, was detected. Subretinal injection of  $1.5 \times 10^{12}$  vg/eye of a precursor AAV2-hRPE65v2 vector product in normal-sighted dogs resulted in ocular inflammation and retinal degeneration histologically in regions exposed to the vector. This dose level is 10-fold higher than the clinical dose level of  $1.5 \times 10^{11}$  vg/eye in the applicant's 'Dosage and Indication' section of the label. The no-observed-adverse-effect-level (NOAEL) in NHPs was  $7.5 \times 10^{11}$  vg/eye, which is 5-fold higher than the proposed clinical dose level specified in the applicant's 'Dosage and Administration' section of the label.

Ocular histopathology suggested that there may be a mild immune response due to re-administration of AAV2-hRPE65v2 sequentially to the same eye of the RPE65 mutant dogs. However, re-administration of AAV2-hRPE65v2 to the same eye is not specified in the applicant's 'Dosage and Administration' section of the label.

Biodistribution (BD) of AAV2-hRPE65v2 in NHPs and a precursor AAV2-hRPE65v2 vector product in normal-sighted dogs was determined out to 3 months following subretinal administration. The highest levels of vector DNA were detected primarily in the intraocular fluids (anterior chamber fluid and vitreous) of vector-injected eyes. Low levels of vector DNA sequence were detected in the optic nerve of the vector-injected eye. Very limited distribution of

low levels of vector DNA to non-ocular tissues (e.g., spleen and liver) was detected. No vector DNA was detected in the gonads.

There was no evidence of a pro-inflammatory T cell response to the RPE65 protein in the NHPs. There were no pro-inflammatory T cell responses (IFN- $\gamma$ ) to the AAV2 capsid, except for one NHP previously exposed to AAV2 vector that developed a CD4+ T cell response. There were no pro-inflammatory T cell responses (IFN- $\gamma$ ) to the AAV2 capsid in any dog and a limited T cell response to human RPE65 observed in RPE65 mutant dogs.

Antibodies to human RPE65 were detected transiently in isolated cases in both dogs and NHPs. Antibodies to the AAV2 capsid were present in the anterior chamber fluid and/or serum of normal-sighted and RPE65 mutant dogs. Antibodies to the AAV2 capsid were not detected in naïve NHPs, but were detected in NHPs previously exposed to AAV2 vector.

Based on the biological attributes of AAV2-hRPE65v2, the current scientific publications, and the pharmacology and toxicology data, studies to evaluate safety pharmacology, developmental and reproductive toxicity, genotoxicity, and carcinogenicity/tumorigenicity were not conducted for AAV2-hRPE65v2.

#### **PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:**

There are no nonclinical deficiencies in the pharmacology/toxicology studies, and there are no outstanding requests for additional nonclinical data. The nonclinical data support the licensure application.

#### **Formulation and Chemistry:**

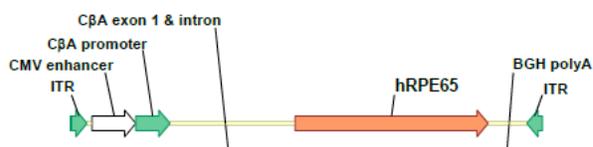
LUXTURNA™ is a solution for injection consisting of  $5 \times 10^{12}$  vector genomes (vg)/mL. The final drug product is a concentrate containing 180 mM sodium chloride, 10 mM sodium phosphate, 0.001% (b) (4) P188 (also known as (b) (4) ), pH 7.3. It is supplied at a volume of 0.5 mL in a 2 mL single-dose vial. The supplied concentration ( $5 \times 10^{12}$  vg/mL) requires a 1:10 dilution with the diluent prior to administration. The diluent, which consists of 180 mM sodium chloride, 10 mM sodium phosphate, 0.001% (b) (4) P188, is supplied in 2 mL single use vials.

The recommended dose level of LUXTURNA™ is  $1.5 \times 10^{11}$  vg in each eye, delivered in a total subretinal volume of 0.3 mL/eye. The administration procedure to each eye should be performed on separate days, no more than 18 days (12 days  $\pm$  6 days) apart.

LUXTURNA™ (also identified as AAV2-hRPE65v2) is a replication-deficient adeno-associated viral (AAV) vector designed to deliver and express the 65 kilodalton (kDa) RPE-specific protein, RPE65. This gene therapy product (schematically presented in Figure 2.6.1; reproduced from the BLA) utilizes a chimeric transcriptional regulatory element comprised of the cytomegalovirus (CMV) enhancer and chicken beta-actin (C $\beta$ A) promoter, for expression of the human RPE65 (hRPE65) gene. The construct also includes the C $\beta$ A non-coding exon 1 and intron 1, which lie upstream of the (b) (4) hRPE65 cDNA and translational start site, and provide signals for mRNA splicing. A bovine growth hormone polyadenylation signal sequence (bGH

poly A) lies downstream of the cDNA. The entire expression cassette is flanked by AAV2 inverted terminal repeats (ITRs).

Figure 2.6.1 Schematic representation of the AAV2-hRPE65v2 vector



Source: Module 2.6.1 in the BLA

### Abbreviations:

AAV	Adeno-associated virus
AC	Anterior chamber
BD	Biodistribution
bGH	Bovine growth hormone
cRPE65	Canine RPE65
cβA	Chicken beta-actin
CMV	Cytomegalovirus
eGFP	Enhanced green-fluorescent protein
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immunosorbent Spot
ERG	Electroretinography
GLP	Good Laboratory Practice
G-W	Goldmann-Witmer
hRPE65	Human RPE65
IFN $\gamma$	Interferon-gamma
IHC	Immunohistochemistry
IOP	Intraocular pressure
ITRs	Inverted terminal repeats
LCA	Leber Congenital Amaurosis
MOI	Multiplicity of infection
NAb	Neutralizing antibody
NHP	Non-human primate
NOAEL	No-observed-adverse-effect-level
PBMC	Peripheral blood mononuclear cells
PR	Photoreceptor
qPCR	Quantitative polymerase chain reaction
RPE	Retinal pigment epithelial
ROA	Route of administration
vg	Vector genome
WT	Wild-type

**Related File(s)**

IND #13408; Spark Therapeutics; A Phase 1 safety study in subjects with Leber Congenital Amaurosis (LCA) using adeno-associated viral vector to deliver the gene for human RPE65 into the retinal pigment epithelium (RPE) [AAV2-hRPE65v2-101]; Treatment of Leber Congenital Amaurosis due to a RPE65 deficiency; ACTIVE

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**INTRODUCTION**

LUXTURN<sup>TM</sup> (AAV2-hRPE65v2) is a gene therapy product indicated for the treatment of patients with vision loss due to confirmed biallelic RPE65 mutation-associated retinal dystrophy. Patients with RPE65 mutations exhibit impaired visual function and progressive photoreceptor (PR) loss. The mechanism of action for AAV2-hRPE65v2 is gene augmentation to express the normal, functional RPE65 protein in affected cells of the retina. The treatment involves sequential bilateral subretinal injections of  $1.5 \times 10^{11}$  vg/eye of AAV2-hRPE65v2.

**NONCLINICAL STUDIES**

**Note:** Per the applicant, during the development of LUXTURN<sup>TM</sup> (AAV2-hRPE65v2) several iterations of the AAV2-RPE65 vector were produced and tested in nonclinical studies (Table 1 below). Both the human (hRPE65) and canine RPE65 (cRPE65) transgenes were cloned into the original vector. To enhance vector safety, the plasmid used to generate the original AAV2-hRPE65 vector was lengthened by insertion of a stuffer sequence into the vector backbone,

creating AAV2-hRPE65v1. However, an inadvertent cloning error occurred within the intron sequence, resulting in decreased expression of the RPE65 protein (see Study #3 in this review memo for details). AAV2-hRPE65v2 was generated to correct this error, and a Kozak sequence was also inserted to further enhance transgene expression.

**Table 1.** Various vector products used in the nonclinical development program for LUXTURNA™

Vector name	Promoter	Intron	Transgene	PolyA	Description
AAV2-hRPE65	Chicken beta actin (C $\beta$ A)	C $\beta$ A exon 1 and intron 1	Human RPE65	Bovine growth hormone polyadenylation signal (bGH)	Original vector encoding human RPE65
AAV2-cRPE65	C $\beta$ A	C $\beta$ A exon 1 and intron 1	Canine RPE65	bGH	Original vector encoding canine RPE65
AAV2-hRPE65v1	C $\beta$ A	C $\beta$ A exon 1 and intron 1	Human RPE65	bGH	Stuffer insertion; Splice acceptor mutation
AAV2-hRPE65v2 (LUXTURNA™)	C $\beta$ A	C $\beta$ A exon 1 and intron 1	Human RPE65	bGH	Stuffer insertion; Splice acceptor mutation corrected; Kozak sequence; (b) (4)

**Note:** Studies not summarized in this review memo included Module 4.2.2 ‘Pharmacokinetics / Module 4.2.2.1 ‘Analytical Methods and Validation Reports’, specifically:

- Enzyme-Linked Immunosorbent Spot (ELISPOT) Assay for the Detection of T Cell Response to Adeno-Associated Virus 2 Capsid and 65KDa Retinal Pigment Epithelium Protein (Study #CHOP-ELISPOT)
- Enzyme-Linked Immunosorbent Assay (ELISA) for Determining Anti-RPE65 Antibody Titers (Study #CHOP-RPE65-ELISA)
- Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Anti-AAV2 Antibody (Study #CHOP-AAV2-ELISA)
- Anti-AAV2 Neutralizing Antibody Detection Assay (Study #CHOP-AAV2-NAB)
- qPCR Analysis for the Evaluation of Biodistribution and Shedding in AAV2-hRPE65 Gene Therapy Studies (Study #CHOP-QPCR)

These analytical methods and validation reports were reviewed by the CMC reviewers and were deemed adequate.

**PHARMACOLOGY STUDIES****Summary List of Pharmacology Studies**

The following pharmacology studies were conducted to support the rationale for the administration of AAV2-hRPE65v2 to treat the proposed clinical indication.

**In Vitro Studies**

<b>Study Number</b>	<b>Study Title / Publication Title</b>	<b>Report Number or Publication Citation</b>
1	Gene Therapy Restores Vision in a Canine Model of Childhood Blindness	Acland G, et al. Nat Genet 2001; 28 (1): 92-95
2	In vitro Bridging Study for LCA Clinical Trial	(b) (4) -in vitro-1
3	AAV2-hRPE65v1 vs AAV2-hRPE65v2 Protein Expression Analysis: A comparison of vector product from two different AAV2-hRPE65 vectors	SPARK-in vitro-1

**In Vivo Studies****In Vivo Studies in Murine Models of RPE65 deficiency**

<b>Study Number</b>	<b>Study Title / Publication Title</b>	<b>Report Number or Publication Citation</b>
4	Reversal of Blindness in Animal Models of Leber Congenital Amaurosis Using Optimized AAV2-Mediated Gene Transfer	Bennicelli J, et al. Mol Ther 2008; 16(3): 458-465

**In Vivo Studies in Canine Model of RPE65 deficiency**

<b>Study Number</b>	<b>Study Title / Publication Title</b>	<b>Report Number or Publication Citation</b>
5	Gene Therapy Restores Vision in a Canine Model of Childhood Blindness	Acland G, et al. Nat Genet 2001; 28 (1): 92-95
6	Long-Term Restoration of Rod and Cone Vision by Single Dose rAAV-Mediated Gene Transfer to the Retina in a Canine Model of Childhood Blindness	Acland G, et al. Mol Ther 2005; 12 (6): 1072-82
7	Safety of Subretinal Administration of AAV2-hRPE65v2 in Affected Canines	(b) (4) Canine Affected-1 or Bennicelli J, et al. Mol Ther 2008; 16(3): 458-465
8	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 (to the Contralateral Eye) in Affected Canines	(b) (4) Canine Contralateral-2 or Amado D, et al. Sci Transl Med 2010; 2(21): 21ra16-21ra16

**Note:** Study Nos. 2-4 and 7-8 are briefly summarized in this review memo under ‘Overview of Pharmacology Studies.’ Study Nos. 1 and 5-6 are not summarized in this review memo because they contained preliminary data generated using earlier versions of the vector product.

## **Overview of Pharmacology Studies**

### *Overview of In Vitro Studies*

#### **Study #2: In vitro Bridging Study for LCA Clinical Trial (Study (b) (4) -in vitro-1)**

##### **Test system:**

- A primary culture of normal canine RPE cells was established from the enucleated eyes of a dog with normal-sighted eyes.
- A primary culture of mutant canine RPE cells was established from the enucleated eyes of an affected (b) (4) dog, which has a naturally occurring mutation (four base-pair deletion) in canine RPE65 that results in the absence of RPE65 protein expression.

**Test article:** AAV2-hRPE65v2

**Control article:** AAV2-eGFP

**Methodology:** Normal and mutant canine RPE cells were transduced with either AAV2-hRPE65v2 or AAV2-eGFP at various MOIs ranging from  $10^2$  to  $10^5$  viral particles (vp)/cell. Cells were harvested at 4 days after transduction.

##### **Results:**

- Quantitative PCR (qPCR) analysis (using primers targeting AAV and hRPE65 transgene) was used to determine the transduction efficiency (vg/cell) of AAV2-hRPE65v2 and the expression of the hRPE65 transgene (cDNA copies/cell). Both AAV2-hRPE65v2 vector genome and hRPE65 transgene were detected (at all MOIs) in AAV2-hRPE65v2 transduced normal and mutant canine RPE cells but not in non-transduced cells or cells transduced with AAV2-eGFP. There was a correlation between the MOI and the transduction efficiency and hRPE65 expression levels.
- The RT-PCR assay was used to measure transgene expression. The hRPE65 mRNA was detected in both normal and mutant RPE cells at all MOIs, but not in non-transduced cells or cells transduced with AAV2-eGFP.
- Immunohistochemistry (IHC) analysis was used to detect hRPE65 protein expression. Transduction of mutant RPE cells with AAV2-hRPE65v2 at MOIs of  $10^5$  and  $10^4$  resulted in strong cytoplasmic staining, while little or no staining was visible at MOIs of  $10^3$  or  $10^2$ . Low intensity, cytoplasmic staining was detected in non-transduced normal RPE cells due to endogenous RPE65 protein expression. Transduction of normal RPE cells with AAV2-hRPE65v2 did not result in increased intensity of RPE65 staining at any MOI when compared with the non-transduced control. No expression of RPE65 protein

was detected in normal and mutant RPE cells transduced with the control vector, AAV2-eGFP.

- There was no increase in the number of apoptotic cells (determined by TUNEL staining) with either normal or mutant RPE cells after transduction with AAV2-hRPE65v2 or AAV2-eGFP at any MOI tested compared to non-transduced cells.

**Summary from the study report:** Transduction of normal or mutant canine RPE cells, using various MOIs, resulted in dose-dependent expression of both hRPE65 mRNA and protein, with no apoptosis observed for the transduced RPE cells.

**Study #3: AAV2-hRPE65v1 vs AAV2-hRPE65v2 Protein Expression Analysis: A comparison of vector product from two different AAV2-hRPE65 vectors (Study SPARK-in vitro-1)**

**Test articles:** AAV2-hRPE65v1; AAV2-hRPE65v2

**Methodology:** HEK293T cells were transduced at various MOIs:  $1 \times 10^4$ ,  $5 \times 10^4$ , and  $1 \times 10^5$ . Cells were harvested at 3 days after transduction and lysed. Protein was extracted for (b) (4) analysis. For some transductions, wild-type (WT) adenovirus at a MOI of 10 was added to the culture media at 2 hours after transduction to enhance protein levels.

**Results:**

- At MOIs of  $5 \times 10^4$  and  $1 \times 10^5$ , a clear RPE65 protein band was visible in lysates from cells transduced with AAV2-hRPE65v2. (b) (4) (i.e. more protein) (b) (4) were observed from lysates of cells co-transduced with AAV2-hRPE65v2 and WT adenovirus. However, no detectable protein was produced from cells transduced with AAV2-hRPE65v1 at any MOI tested, with or without boosting adenoviral transduction. Flow cytometric analysis confirmed these results.

**Summary from the study report:** AAV2-hRPE65v2 resulted in dose-dependent RPE65 expression in HEK293T cells as assessed by both (b) (4) analyses. In contrast, no detectable hRPE65 protein was observed following transduction of cells with AAV2-hRPE65v1 at all three MOIs. These data confirm the error that occurred in the intron sequence during the cloning of AAV2-hRPE65v1, which resulted in decreased expression of the RPE65 protein (see Table 1 in this review memo).

Overview of In Vivo Studies

**In Vivo Studies in Murine Models of RPE65 deficiency**

**Murine Model #1:**

The *RPE65*<sup>-/-</sup> murine model was generated by a targeted disruption of mouse RPE65. Retinal function is severely abnormal in these mice by 3 to 4 months of age, and the mice develop progressive retinal degeneration, with changes in retinal physiology and biochemistry exhibited

by 15 weeks of age. Outer segment discs of rod photoreceptors (PRs) are disorganized and rod function, as measured by ERG, is abolished<sup>1</sup>.

### **Murine Model #2:**

The *rd12* mice are a naturally occurring murine model of RPE65 gene mutation that was identified by screening mice using indirect ophthalmoscopy and ERG. The genetic defect was identified as a nonsense mutation in exon 3 of the RPE65 gene, which is predicted to result in loss of function of the protein. These mice exhibit diminished ERG responses as early as 3 weeks of age, and develop retinal degeneration at approximately 3 months of age<sup>2</sup>.

### **Study #4: Reversal of Blindness in Animal Models of Leber Congenital Amaurosis Using Optimized AAV2-Mediated Gene Transfer**

**Test article:** AAV2-hRPE65v2

*Experiment in RPE65<sup>-/-</sup> mice:*

#### **Study design:**

Unilateral subretinal injections of AAV2-hRPE65v2 at  $1 \times 10^9$  vg/eye (1  $\mu$ L volume) were performed in *RPE65<sup>-/-</sup>* mice (n=10; aged 1-2 months). Contralateral eyes were subjected to a sham injection. ERGs were performed to identify any improvement in retinal function at 3-4 weeks after injection. Animals were then sacrificed and the eyes were harvested immediately.

#### **Results:**

- Waveforms of the sham-injected eyes were essentially flat. In contrast, there were improved waveforms and amplitudes in the AAV2-hRPE65v2-injected eyes in 4 of the 10 mice.
- Improvements were observed in both the scotopic  $b_{\max}$  and the rod and cone PR  $a_{\max}$ :

<b>RPE65<sup>-/-</sup> mice</b>	<b>Scotopic <math>b_{\max}</math> (<math>\mu</math>V)</b>	<b>Rod and cone PR <math>a_{\max}</math> (<math>\mu</math>V)</b>
Sham-injected	0.5	0
AAV2-hRPE65v2 injected	59	189
WT C57BL/6	233	567

- Results from IHC showed the presence of RPE65 protein in RPE cells in eyes injected with AAV2-hRPE65v2. There was no detectable RPE65 protein in the RPE cells of a sham-injected *RPE65<sup>-/-</sup>* mouse retina.

<sup>1</sup> Redmond TM, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nat Genet 1998; 20: 344-51.

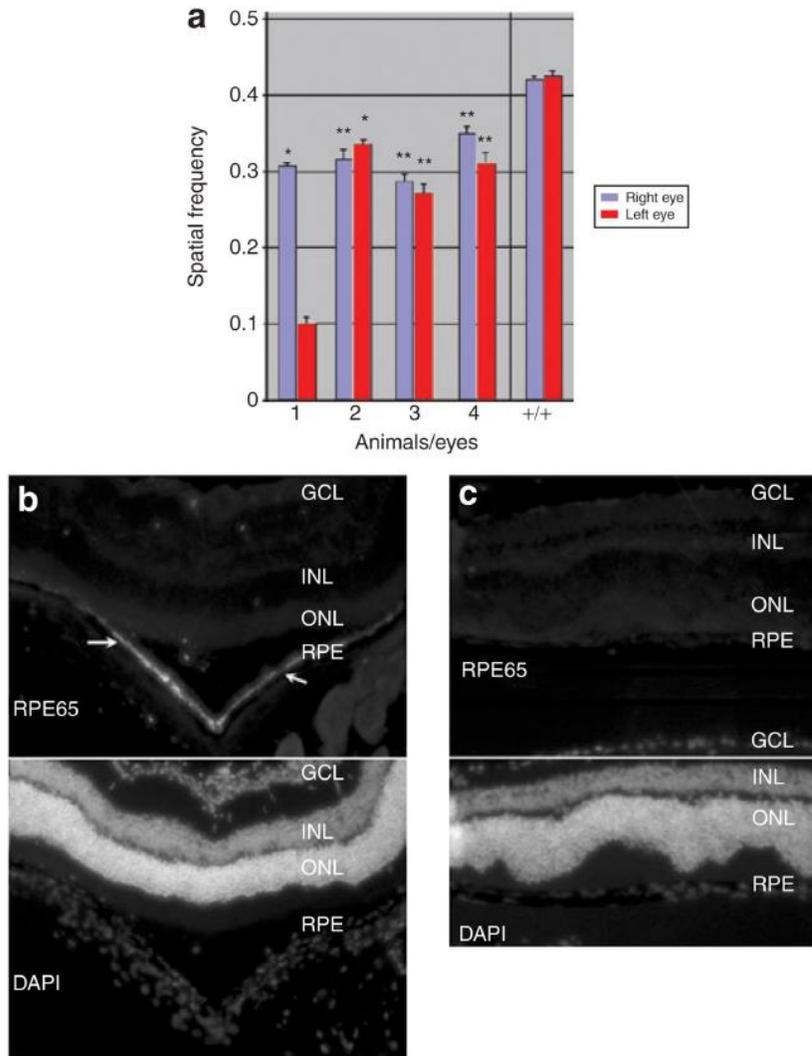
<sup>2</sup> Pang JJ, et al. Retinal degeneration 12 (rd12): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). Mol Vis 2005; 11: 152-62.

*Experiment in rd12 mice:***Study design:**

Bilateral subretinal injections of AAV2-hRPE65v2 at  $1 \times 10^9$  vg/eye (1  $\mu$ L volume) were performed in three *rd12* mice (Animal #s 2-4; 1-2 months of age). A unilateral (right eye) subretinal injection of  $1 \times 10^9$  vg/eye (1  $\mu$ L volume) was performed in one *rd12* mouse (Animal #1); the contralateral left eye was subjected to a sham injection. ERG and visual acuity were measured one month post-injection. The visual acuity in both eyes of the four *rd12* mice was measured using optomotor responses to a rotating sine-wave grating.

**Results:**

- Similar reversals of ERG deficits were observed in the *rd12* mice as in the *RPE65*<sup>-/-</sup> mice.
- Improvements in visual acuity were observed in all eyes injected with AAV2-hRPE65v2 (i.e., both eyes of Animals #2-4 and the right eye of Animal #1) compared to the sham-injected left eye of Animal #1 (Figure 2a; reproduced from the BLA).
- RPE65 protein was detected in the RPE cells, but not other retinal cell types, in the injected eyes (Figure 2b; reproduced from the BLA). RPE65 protein was absent in the sham-injected eye (Figure 2c; reproduced from the BLA).



**Figure 2. Improvement in visual acuity in mice correlates with immunohistochemical localization of RPE65 in Rpe cells after sub-retinal injection with AAV2-RPE65**

(a) Post-treatment optokinetic (Optomotry) responses of treated and control eyes of four *rd12* (*Rpe65* null) mice and of a control (phenotypically normal) C57Bl/6 mouse. There is significant improvement in visual acuity in eyes of all *rd12* mice injected subretinally with AAV2-RPE65 compared with the control eye (left eye of animal 1). \* $P \leq 0.005$ ; \*\* $P \leq 0.02$ . Testing was performed 1 month post-injection. The experimenter was unaware of the age, and treatment paradigm of the tested animals and the direction of rotation of the sinusoidal pattern presented to the animal. Mice were tested under photopic conditions during their daytime light cycle and four separate trials were performed per animal. RPE65 protein is detectable by immunohistochemistry in (b) the rpe (only) of the right, subretinally injected eye, but not in (c) the contralateral, uninjected (control) eye. These are the eyes from animal #1, shown in a. Upper panels in b and c show RPE65-specific fluorescence (arrows); lower panels show 4,6-diamidino-2-phenylindole (DAPI) fluorescence. Immunohistochemistry was performed 1 month post-injection. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium.

**Source:** Bennicelli J, et al. Reversal of Blindness in Animal Models of Leber Congenital Amaurosis Using Optimized AAV2-Mediated Gene Transfer *Mol Ther* 2008; 16(3): 458-465

**Publication summary:**

Improved ERG and visual acuity were observed in murine models of RPE65 deficiency. Consistent with the functional improvements, transduced RPE65 protein was identified in RPE cells only in eyes injected with AAV2-hRPE65v2.

***In Vivo* Studies in a Canine Model of RPE65 Deficiency****Animal model:**

Some (b) (4) dogs represent a naturally occurring canine model of RPE65 deficiency due to a homozygous mutation in the cRPE65 gene. A deletion of four base pairs in the gene results in a frameshift and a premature stop codon, with subsequent absence of RPE65 protein expression. These dogs show disease phenotypes similar to those seen in humans with inherited retinal degeneration due to autosomal recessive RPE65 gene mutations, including congenital visual dysfunction, followed later by retinal degeneration<sup>3</sup>.

**Study #7:**

<b>Report Number</b>	(b) (4) Canine Affected-1	
<b>Date Report Signed</b>	June 27, 2012	
<b>Title</b>	Safety of Subretinal Administration of AAV2-hRPE65v2 in Affected Canines	
<b>GLP Status</b>	No	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the safety of administration of AAV2-hRPE65v2 to affected ((b) (4) ) dogs via subretinal injection	
<b>Study Animals</b>	<b>Animal Model</b>	RPE65-/- (b) (4) dogs
	<b>Age</b>	3.5 months
	<b>Body Weight</b>	Not provided
	<b>#/sex (animal ID)</b>	1M (#BR329)/2F (#BR334 and #BR332)
<b>Total #</b>	3	
<b>Test Article(s)</b>	AAV2-hRPE65v2 formulated in PBS, with or without 0.001% Pluronic (b) (4)	
<b>Control Article(s)</b>	N/A	
<b>Route of Administration</b>	Subretinal or intravitreal injection	
<b>Study Groups and Dose Levels</b>	See Table 1 below (reproduced from the study report)	
<b>Dosing Regimen</b>	Bilateral, simultaneous single injections	
<b>Randomization</b>	No	
<b>Description of Masking</b>	Not provided	
<b>Scheduled Sacrifice Time Points</b>	5 weeks (#BR329) and 3 months (#BR334 and BR332) post-injection	

<sup>3</sup> Narfstrom K, et al. The Briard Dog: A New Animal Model of Congenital Stationary Night Blindness. Br J Ophthalmol 1989; 73: 50-56

Table 1: Dose Levels and Schedule

Animal/ID	Dose Level (Subretinal) in Right Eye, 8/29/06 (Day 0) (vg)	Dose Level (Subretinal) in Left Eye, 8/29/06 (Day 0) (vg)
1. BR329 (male)	8.25E10 / PBS180 Subretinal	8.25E10 / PBS180/PF68 Subretinal
2. BR334 (female)	8.25E10 / PBS180/PF68; Intravitreal	8.25E10 / PBS180; Subretinal
3. BR 332 (female)	8.25E10 / PBS180/PF68 Subretinal	8.25E10 / PBS180/PF68 Subretinal

**Source:** Study Report #(b) (4) Canine Affected-1, submitted in Module 4.2.3.1 of the BLA

### *Key Evaluations and Assessments:*

#### In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Daily
- Ophthalmoscopic examination: Prior to injection and on days 3, 7, and 14 post-injection
- Navigation<sup>4</sup>, nystagmus, pupillometry (prior to injection and weekly for 5 weeks post-injection)
- ERGs: 5 weeks (for all dogs) and 3 months (for #BR334 and #BR332) post-injection
- Enzyme-Linked Immunosorbent Assay (ELISA) for antibody response to RPE65 protein and AAV2 capsid (serum and anterior chamber fluid): baseline and 5 weeks (for all dogs) and 3 months (for #BR334 and #BR332)
- Neutralizing antibodies (NAbs) to AAV2 capsid (serum and anterior chamber fluid): baseline and 5 weeks (for all dogs) and 3 months (for #BR334 and #BR332)
- Enzyme-Linked Immunosorbent Spot (ELISPOT) assay for inflammatory cytokine response to AAV2 capsid [secretion of IFN $\gamma$  by peripheral blood mononuclear cells (PBMCs)]: 3 months only (for #BR334 and #BR332)

#### Terminal:

- Gross pathology
- Ocular histopathology
- Immunofluorescence analysis for identification of RPE65 protein

#### *Key Results:*

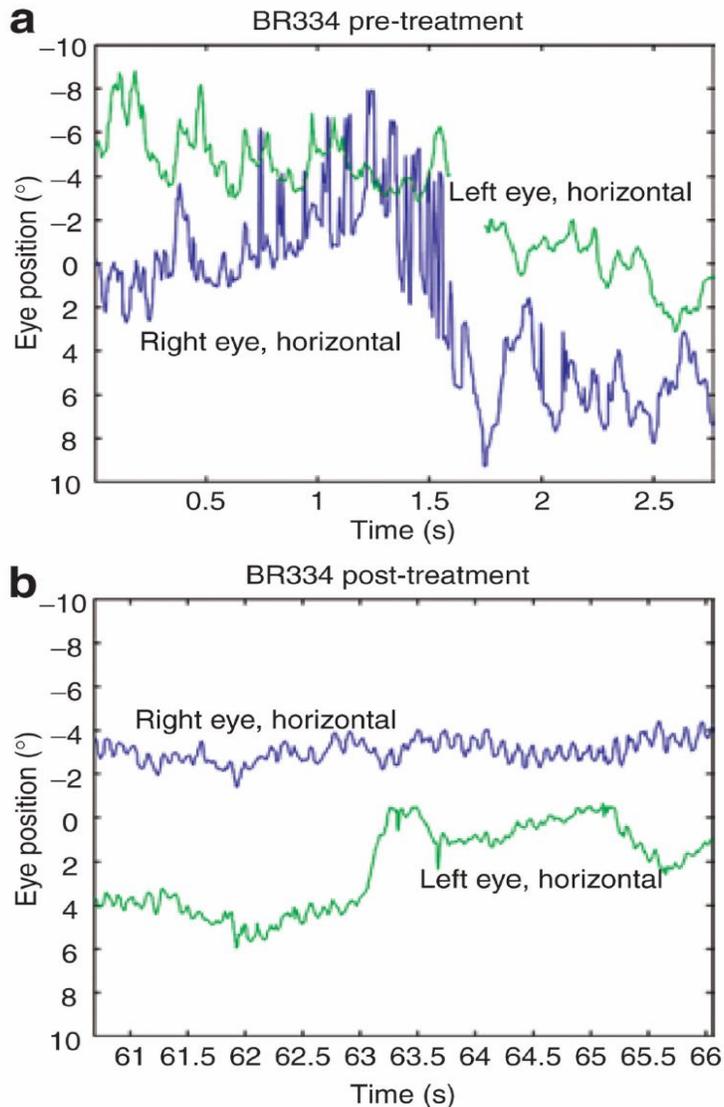
- There were no unscheduled deaths during the study.
- There were no abnormal clinical observations.
- Ophthalmoscopic examination: There was an inadvertent choroidal injury during injection of the right eye of #BR332 that resulted in an intravitreal hemorrhage (**Note:**

<sup>4</sup> Navigation evaluation included observing the ability of the animals to avoid obstacles in their paths and move from one point to the next with confidence.

there was no inflammation in this eye based on the ocular histopathology examination). There were no other test article-related or surgery-related injuries or signs of toxicity noted in the other animals at all follow-up examinations.

- **Navigation:** Prior to injection, each animal was timid and moved slowly. The animals would bump into obstacles that were in their path (including doors to their runs). Within 2 weeks after the injection, animals showed improved navigation, which continued to improve for each animal throughout the duration of the study.
- **Nystagmus:** Before vector injection, nystagmus was present in both eyes of #BR334, consisting of large amplitude and lower frequency, predominantly the jerk waveform. By one month post-injection, nystagmus was reduced; specifically, the jerk component was eliminated, and the amplitude of the nystagmus in the left (subretinally injected) eye was half of that in the right eye (intravitreally injected) (Figure 3).

**Note:** Nystagmus data for Animal #BR334 only were included in the study report.



**Figure 3. Nystagmus wave forms of both eyes of dog BR334 (a) before and (b) 4 weeks after subretinal injection in the left eye**

**a** Nystagmus is present in both eyes with comparable characteristics, consisting of a large amplitude ( $\sim 4^\circ$  peak-to-peak, p-p), lower-frequency ( $\sim 3$  Hz) predominantly jerk waveform combined with a smaller amplitude ( $\sim 1\text{--}2^\circ$  p-p), high-frequency ( $\sim 10$  Hz) pendular oscillation.

**b** One month post-treatment, the jerk component has been eliminated, leaving only the underlying pendular oscillation. At times, the amplitude of the nystagmus in the left (subretinally injected) eye is approximately half of that in the right eye. This study presents additional evidence of the strong yoking between the movements of the two eyes, with smooth pursuit having dominance over nystagmus.

**Source:** Bennicelli J, et al. Reversal of Blindness in Animal Models of Leber Congenital Amaurosis Using Optimized AAV2-Mediated Gene Transfer *Mol Ther* 2008; 16(3): 458-465

- Pupillometry: Pupillary light reflexes improved in the eyes injected subretinally with AAV-hRPE65v2, but not in the one eye injected intravitreally with vector (#BR334).

- ERGs: Prior to injection, all dogs showed a flat ERG response. Post-injection, all subretinally injected eyes showed a moderate ERG response (e.g., increased amplitudes in the waveforms) and improved light sensitivity at all time points tested. The best ERG responses were found in eyes injected subretinally with AAV2-hRPE65v2 containing (b) (4) (i.e., both eyes of #BR332), suggesting improved vector delivery in the presence of this surfactant.  
**Note:** (b) (4) is included in the final formulation for LUXTURNA™.
- ELISA (serum and anterior chamber fluid):
  - There was no specific antibody response to the human RPE65 transgene product in the anterior chamber fluid or serum samples.
  - The serum samples for all three dogs contained antibodies (titer of 1:1000) to the AAV2 capsid at the 5-week time point. However, no antibodies were detected at the 3-month time point in the two surviving animals for which serum samples were collected.
  - There was no detectable antibody response to AAV2 capsid in anterior chamber fluid samples from either eye at the 5-week (#BR329) or the 3-month (#BR332 and #BR334) time points.
- NABs to AAV2 capsid (serum and anterior chamber fluid):
  - No NABs were detected in any of the baseline serum samples. NABs were detected at a titer of 1: 1000 in 2/3 animals at the 5-week time point, but decreased to a titer of 1:10 in both surviving dogs at the 3-month time point.
  - No NABs were detected in the anterior chamber fluid (collected at sacrifice) at 5 weeks post-injection (#BR329). The fluid from #BR332 and #BR334 had low titers of 1:10 and 1:5, respectively, at 3 months post-injection.
- ELISPOT analysis: There were no detectable cytokine responses to the AAV2 capsid in PBMCs collected from the three animals.
- Gross pathology: There were no abnormal gross pathology findings.
- Ocular histopathology:
  - Abnormalities consistent with RPE65 deficiency were identified, including RPE vacuolation, RPE pigment clumping, RPE atrophy and hypertrophy, subretinal microgliosis, and modest multifocal atrophy of the outer nuclear layer (ONL). The RPE65 deficiencies had not progressed at 3 months compared to 5 weeks post-injection.
  - The following mild abnormalities were noted: retinal atrophy and scarring, focal retinal detachment, RPE loss, RPE hypertrophy, modest subretinal microgliosis, and PR atrophy. These lesions were determined to be related to the injection procedure by the pathologist.

**Note:** The study report provided minimal information about the eye that was injected intravitreally.

- There was no evidence of inflammation.
- Preservation of the ONL and of PR outer segments was observed in the subretinal-injected regions.

**Note:** The study report did not specify the outcome for the eye that was intravitreally injected.

- Immunofluorescence: RPE65 protein was present only in the RPE cells of the injected region of the retina. Minimal presence of RPE65 protein in the optic nerves and ganglion cells was detected in eyes injected subretinally.

#### Summary from the study report:

- Improvement of visual function was observed as early as 2 weeks post-injection.
- There were no significant inflammatory or other adverse ophthalmic effects associated with the test article or the formulation.
- The RPE cells in the injected region expressed the human RPE65 protein.
- There were transient increases (compared to baseline) in humoral (but not intraocular) anti-AAV2 capsid antibodies post-injection; the titers decreased by 3 months.
- There was no evidence of cytokine response to the AAV2 capsid.

#### Study #8:

**Note:** This pharmacology study also evaluated safety endpoints following bilateral, sequential subretinal injection of AAV2-hRPE65v2.

<b>Report Number</b>	(b) (4) Canine Contralateral-2	
<b>Date Report Signed</b>	June 27, 2012	
<b>Title</b>	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 (to the Contralateral Eye) in Affected Canines	
<b>GLP Status</b>	No	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the safety of re-administration of AAV2-hRPE65v2 when administered to the contralateral eye of affected (b) (4) dogs via subretinal injection	
<b>Study Animals</b>	<b>Animal Model</b>	RPE65-/- (b) (4) dogs
	<b>Age</b>	One year old
	<b>Body Weight</b>	28-31 kg at dosing
	<b>#/sex</b>	4M/2F
	<b>Total #</b>	6
<b>Test Article(s)</b>	AAV2-hRPE65v2	

<b>Excipient of test article</b>	180mM Sodium Chloride/10mM Sodium Phosphate pH 7.3, 0.001% Pluronic <sup>(b) (4)</sup>
<b>Route of Administration</b>	Subretinal injection
<b>Study Groups and Dose Levels</b>	See Table 1 below (reproduced from the study report)
<b>Dosing Regimen</b>	Bilateral, sequential [left eye injected approximately two weeks after the right eye]
<b>Randomization</b>	No
<b>Description of Masking</b>	Not provided
<b>Scheduled Sacrifice Time Point</b>	Approximately 2 years post-first-eye injection <b>Note:</b> One dog, Venus, was not sacrificed, but was transferred to Study #14.

**Table 1. Subretinal Injection Dates for Bilateral Re-Administration Study in Affected Dogs**

Animal ID	Right Eye	Left Eye
Remy (male; D07-7)	Subretinal 1.5E11 vg (8/14/07)	Subretinal 1.5E11 vg (8/28/07)
Nixie (female; D06-296)	Subretinal 1.5E11 vg (8/14/07)	Subretinal 1.5E11 vg (8/27/07)
Sebby (male; D07-5)	Subretinal 1.5E11 vg (8/14/07)	Subretinal 1.5E11 vg (8/29/07)
Venus (female; D06-295)	Subretinal 1.5E11 vg (8/14/07)	Subretinal 1.5E11 vg (8/28/07)
Percy (male; D07-6)	Subretinal 1.5E11 vg (8/27/07)	Subretinal 1.5E11 vg (9/9/07)
Neptune (male; D06-294)	Subretinal 1.5E11 vg (8/27/07)	Subretinal 1.5E11 vg (9/9/07)

**Source:** Study Report <sup>(b) (4)</sup> Canine Contralateral-2, submitted in Module 4.2.3.2 of the BLA

**Comment:**

- The dose level and dosing schedule in this study are identical to those evaluated in the Phase 3 clinical trial.

*Key Evaluations and Assessments:*

In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Once daily
- Body weights (BWs): multiple time points post-injection
- Ophthalmic examinations: Prior to first-eye injection and on days 3, 7, 14-15, and 28-43 and 13-14.5 months post-first-eye injection, and prior to sacrifice
- Navigation, nystagmus, pupillary light reflexes: Prior to injection, weekly for 6 weeks post-injection, prior to sacrifice
- ELISA for antibody response to RPE65 protein and AAV2 capsid (serum and anterior chamber fluid): Prior to first-eye injection and multiple time points post injection
- NAb to AAV2 capsid (serum and intraocular fluids): Prior to injection and multiple time points post-injection
- ELISPOT assay for cytokine response (IFN $\gamma$  and IL-10 in PBMCs) to AAV2 capsid and RPE65 protein: Multiple time points post-injection

**Terminal:**

- Gross pathology
- Ocular histopathology
- Immunofluorescence analysis for identification of RPE65 protein

***Key Results:***

- There were no unscheduled deaths during the study.
- There were no abnormal clinical observations, BWs, or ophthalmoscopic examination findings.
- Navigation: Prior to injection, each animal was timid and moved slowly. The animals would bump into obstacles that were in their path (including doors to their runs). Within 2 weeks after injection of the first eye, animals showed improved navigation. Navigation continued to improve after the second eye was injected. These improvements persisted for approximately two years after the first injection (the end of the study).
- Nystagmus: There were no large excursions of eye movements prior to, or following subretinal injection of AAV2-hRPE65v2 except for the male dog named Remy. At baseline, Remy had high frequency (3-5 Hz) nystagmus in both eyes; by 3 weeks after injection (**Note:** the study report did not specify whether the 3-week time point was relative to the first- or second-eye injection), the frequency of the nystagmus diminished to 1 Hz. There was no detectable nystagmus in any of the animals (including Remy) at the end of the study.
- Pupillary light reflexes: Improved pupillary light reflexes were noted in each injected eye.
- ELISA:
  - Anti-AAV2 antibody: First-eye administration and re-administration of AAV2-hRPE65v2 into the contralateral eye resulted in an increase in serum antibodies to the AAV2 capsid in samples collected on days 15 and 45 post-first-eye injection and at sacrifice. Antibody subtyping showed an IgM and IgG2 (but not IgG1) response to the AAV2 capsid, suggesting a primary Th2 response to the AAV2 capsid.
  - Anti-RPE65 antibody: Serum anti-RPE65 antibodies were detected with a titer of 1:1000, in the animal Venus at baseline (prior to first-eye injection) and one month after second-eye injection. All other samples (serum, anterior chamber fluid) from Venus were negative. All samples from the other animals were negative at all time points.

**Comment:**

- It was not clear why anti-RPE 65 antibodies were detected at baseline in the animal Venus.

- NABs to AAV2 capsid: Table 1 (reproduced from the study report)
  - Serum: No NABs were detected in serum collected before AAV2-hRPE65v2 administration. The titer increased in all animals at days 15 and 45 post-first-eye injection. The titers decreased over time in all animals.
  - Intraocular fluids: No NABs were detected in samples collected before AAV2-hRPE65v2 administration. The NAb titers in the anterior chamber increased post-injection and remained elevated in five dogs at the time of sacrifice (>20 months post-injection). The ratio of antibodies in the anterior chamber to that in the serum (Goldmann-Witmer (G-W) ratio) was elevated in 5 of the 10 eyes analyzed. For these five dogs, the vitreous fluid (which could be collected only at termination of the study) contained higher NAb titers than in the anterior chamber fluid or in the serum collected at sacrifice.  
**Note:** One dog (Venus) was not sacrificed.

**Note:** The G-W ratio is a calculation that determines the intraocular production of antibodies, and is based on comparing the level of specific antibodies to total immunoglobulin in both aqueous humor and serum. An elevated G-W ratio may indicate that the antibodies detected in the eye are the result of local production rather than leakage of antibodies from the circulation through the blood-aqueous barrier<sup>5</sup>.

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<sup>5</sup> Van der Voet JC, et al. Intraocular antibody synthesis during experimental uveitis. *Investigative Ophthalmology & Visual Science* 1989; 30: 316.

**Table 1.** AAV2 NAb titers in affected dogs. Day, days after first injection; day 0, baseline measurement; gray boxes, no sample collected; NA, not analyzed; ND, not done (animal is still under study).

Animal ID	Sample	Day 0	Day 15	Day 45	23.5 to 27 months	G-W ratio (terminal)
		Right eye injected	Left eye injected	Serum collected	Necropsy	
RM	Serum	<1:3.16	1:10–1:31.6	1:1,000–1:3,160	1:316–1:1,000	
	Left AC		NA		1:100–1:316	0.3
	Right AC	<1:3.16			1:100–1:316	0.3
	Left vitreous				>3,160	
	Right vitreous				>3,160	
NY	Serum	NA	<1:3.16	1:316–1:1,000	1:31.6–1:100	
	Left AC		<1:3.16		1:316–1:1,000	10.0
	Right AC	<1:3.16			1:316–1:1,000	10.0
	Left vitreous				>1:3,160	
	Right vitreous				>1:3,160	
NE	Serum	<1:3.16	1:10–1:31.6	1:100–1:316	1:31.6–1:100	
	Left AC		<1:3.16		1:316–1:1,000	10.0
	Right AC	<1:3.16			1:316–1:1,000	10.0
	Left vitreous				1:1,000–1:3,160	
	Right vitreous				1:1,000–1:3,160	
SE	Serum	<1:3.16	1:31.6–1:100	1:1,000–1:3,160	1:100–1:316	
	Left AC		NA		1:31.6–1:100	0.3
	Right AC	NA			1:31.6–1:100	0.3
	Left vitreous				1:1,000–1:3,160	
	Right vitreous				>3,160	
VE	Serum	<1:3.16	<1:3.16	1:3,160–1:10,000	1:316–1:1,000	
	Left AC	NA	<1:3.16		ND	
	Right AC	NA			ND	
	Left vitreous				ND	
	Right vitreous				ND	
PE	Serum	<1:3.16	1:3.16–1:10	1:31.6–1:100	1:10–1:31.6	
	Left AC		<1:3.16		1:3.16–1:10	0.3
	Right AC	<1:3.16			1:100–1:316	10.0
	Left vitreous				1:31.6–1:100	
	Right vitreous				>1:3,160	

**Source:** Amado D, et al. Safety and Efficacy of Subretinal Readministration of a Viral Vector in Large Animals to Treat Congenital Blindness. *Sci Transl Med* 2010; 2(21): 21ra16-21ra16

- ELISPOT analysis: No cytokine responses to either the AAV2 capsid or the RPE65 transgene product were detected in any dog at any time point after the first-eye or the second-eye injection (2 weeks and 1, 1.5, 5, and 6 months after the first-eye injection).
- Gross pathology: There were no extra-ocular abnormalities detected in any of the dogs.
- Ocular histopathology:
  - Disappearance of lipid vacuoles was observed in the RPE cells of the injected region of the retina. Vacuoles were present in RPE cells of the uninjected regions of the retina.

**Note:** Per the study report, RPE vacuoles are thought to contain a build-up of retinyl ester due to RPE65 deficiency. Delivery of the WT RPE65 cDNA allows these esters to be broken down and subsequent vacuole disappearance.

- Isolated foci of inflammatory cells (predominantly intravitreal) were observed in both eyes. Per the pathologist, this finding may be a mild immune response due to re-administration of the test article to the contralateral eye.
- Per the pathologist, the following findings noted at the sites of injection were most likely due to the surgical procedure: occasional hypertrophy and clumping of RPE cells, focal loss of PR cells, rosette formation, presence of a few macrophages, occasional subtle RPE abnormalities (including loss of pigment granules), and a few detached RPE cells.
- Immunofluorescence: RPE65 protein expression was detected in the injected region of the retina in both eyes, suggesting that re-administration to the contralateral eye did not prevent gene transduction in the first eye. Expression of RPE65 protein was not detected in the ganglion cell axons in the optic nerve. Minimal RPE65 expression was detected in the PRs.

#### **Summary from the study report:**

- Visual behavior of the RPE65 mutant dogs improved following AAV2-hRPE65v2 administration and re-administration into the contralateral eye. This visual behavior was maintained for the duration of the study.
- A mild immune response was noted in both eyes at sacrifice due to administration of AAV2-hRPE65v2 to the contralateral eye. The findings were asymptomatic and did not cause an apparent reduction in transduction or in visual behavior of the animals.
- Subretinal administration of AAV vector into one eye of RPE65 mutant dogs, followed by administration approximately 2 weeks later, into the second eye, did not result in T cell responses to the AAV2 capsid or to the human RPE65 transgene product.

### **SAFETY PHARMACOLOGY STUDIES**

#### **Summary List of Safety Pharmacology Studies**

No safety pharmacology studies were conducted.

### **PHARMACOKINETIC STUDIES (Biodistribution)**

#### **Summary List of Pharmacokinetic Studies**

Determination of vector BD profiles was incorporated in the toxicology studies. These data are summarized with the respective toxicology study.

## TOXICOLOGY STUDIES

### Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety and BD of AAV2-hRPE65v2 following administration in normal-sighted and RPE65 mutant dogs and normal-sighted NHPs. Both single and repeat administrations were evaluated.

Single administration: Two dosing regimens were used: 1) single injection, unilateral administration (Studies #9 and #10) and 2) single injection, bilateral, simultaneous administration (Study #11).

Repeat administration: Three dosing regimens were used:

- a. Administration to one eye, followed (at a later interval) by administration to the contralateral eye (bilateral, sequential; Studies #8 and #12).  
**Note:** This dosing regimen reflects the clinical regimen ('Dosage and Administration' in the label).
- b. Administration to one eye, followed (at a later interval) by administration to the contralateral eye, then re-administration to one of the eyes (bilateral, sequential/re-administration; Study #13).
- c. Administration to both eyes, followed (at a later interval) by re-administration to one eye (bilateral, simultaneous/re-administration; Study #14).

### **Comment:**

- o Based on the transcript and summary minutes from the FDA Cellular, Tissue and Gene Therapies Advisory Committee meeting held on June 29, 2011 to discuss cellular and gene therapy products for the treatment of retinal disorders (<http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/CellularTissueandGeneTherapiesAdvisoryCommittee/ucm249846.htm>), safety data generated from first-eye administration of a gene therapy product in humans may be a more relevant assessment to support second-eye administration than data generated from animal study.

### Toxicology Studies:

Study Number	Study Title / Publication Title	Report Number or Publication Citation
8	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 (to the Contralateral Eye) in Affected Canines	(b) (4) Canine Contralateral-2 and Amado D, et al. Sci Transl Med 2010; 2(21): 21ra16-21ra16
9	Three-Week Toxicity Study of AAV2/2.CBA.hRPE65 Following a Single Subretinal or Intravitreal Injection in (b) (4) Dogs	0406DC93.001
10	Three-Month Toxicity Study of AAV2/2.CBA.hRPE65 Following a Single Subretinal or Intravitreal Injection in (b) (4) Dogs	0470DC93.001

Study Number	Study Title / Publication Title	Report Number or Publication Citation
11	Three-Month Toxicity Study of AAV2/2.CBA.hRPE65v2 Following a Single Subretinal Injection in (b) (4) Monkeys	XHB-00009
12	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 in Non-Human Primates	(b) (4) NHP-5 and Amado D, et al. Sci Transl Med 2010; 2(21): 21ra16-21ra16
13	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 in Unaffected Canines	(b) (4) Canine Unaffected-4
14	Safety of Ipsilateral Subretinal Re-Administration of AAV2-hRPE65v2 in Affected Canines	(b) (4) Canine Ipsilateral-3

Developmental and Reproductive Toxicology Studies:

Studies were not conducted to evaluate this safety endpoint because:

- Subretinal administration of AAV2-hRPE65v2 is associated with limited systemic distribution as shown in the BD data in animals.
- The presence of AAV2-hRPE65v2 was not detected by qPCR in the gonads of healthy NHPs following subretinal administration.
- AAV vectors do not transduce mature sperm<sup>6</sup>.
- A possible causal role of AAV in the occurrence of miscarriage was mentioned in the BLA because of the finding that wild-type (wt)AAV2 interferes with mouse embryonic development. In brief, *in vitro* infection of fertilized mouse oocytes with wtAAV2 resulted in growth arrest at the two-cell stage. Further research showed that a small DNA sequence containing the P5 promoter induced developmental arrest<sup>7</sup>. Since this sequence is absent from recombinant AAV vector genomes, this finding is unlikely to be relevant to AAV2-hRPE65v2.

<sup>6</sup> Couto L, et al. Direct Exposure of Mouse Spermatozoa to Very High Concentrations of a Serotype-2 Adeno-associated Virus Gene Therapy Vector Fails to Lead to Germ Cell Transduction. Hum Gene Ther. 2004; 15:287–291.

<sup>7</sup> Botquin V, et al. Adeno-associated Virus Type 2 Interferes with Early Development of Mouse Embryos. J Gen Virol. 1994; 75: 2655–2662.

Genotoxicity Studies:

Studies were not conducted to evaluate this safety endpoint because:

- The current scientific literature reports a low integration frequency of AAV vectors and integration profile of AAV vectors (e.g., no preferential integration)<sup>8,9,10</sup>.
- The vector BD profile following subretinal administration in normal-sighted eyes of healthy NHPs and dogs showed limited systemic exposure of the vector.

Carcinogenicity/Tumorigenicity Studies:

Studies were not conducted to evaluate this safety endpoint because:

- AAV2-hRPE65v2 is administered to post-mitotic retinal tissue. AAV vectors do not encode proteins that catalyze integration, but rather rely on cellular factors to integrate into pre-existing chromosomal breaks<sup>11</sup>. Thus, the risk for AAV vector-induced insertional mutagenesis/carcinogenesis, particularly in post-mitotic cells such as retinal cells, is considered low.
- Clonal integration of wtAAV2 into known cancer-causing genes was found in 11 of 193 human hepatocellular carcinomas<sup>12</sup>, suggesting a possible role of wtAAV2 in liver carcinogenicity by insertional mutagenesis. However, most of the integration events included the 3' portion of the wtAAV2 capsid gene, which is absent in AAV2 vectors. Thus, the relevance of this finding to recombinant AAV2 vectors is unclear.
- No evidence exists from nonclinical studies showing that subretinal administration of AAV2-hRPE65v2 results in systemic toxicity or abnormal cell proliferation.
- Ongoing clinical trial (under IND #13408) since October 2007 has shown no evidence of tumor formation clinically in any subjects receiving AAV2-hRPE65v2.

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<sup>8</sup> Nowrouzi A, et al. Integration Frequency and Intermolecular Recombination of rAAV Vectors in Non-human Primate Skeletal Muscle and Liver. *Mol Ther.* 2012; 20: 1177–1186.

<sup>9</sup> Kaeppl C, et al. A Largely Random AAV Integration Profile after LPLD Gene Therapy. *Nat Med.* 2013; 19: 889–891.

<sup>10</sup> Huser D, et al. Adeno-associated Virus Type 2 Wild-type and Vector-mediated Genomic Integration Profiles of Human Diploid Fibroblasts Analyzed by Third-generation PacBio DNA Sequencing. *J Virol.* 2014; 88: 11253–11263.

<sup>11</sup> Miller DG, et al. Adeno-associated Virus Vectors Integrate at Chromosome Breakage Sites. *Nat Genet.* 2004; 36: 767–773.

<sup>12</sup> Nault J, et al. Recurrent AAV2-related Insertional Mutagenesis in Human Hepatocellular Carcinomas. *Nat Genet.* 2015; 47: 1187–1193.

## Toxicology Studies

### Study #9

<b>Report Number</b>	0406DC93.001	
<b>Date Report Signed</b>	June 25, 2009	
<b>Title</b>	Three-Week Toxicity Study of AAV2/2.CBA hRPE65 Following a Single Subretinal or Intravitreal Injection in (b) (4) Dogs	
<b>GLP Status</b>	Yes	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the acute toxicity of AAV2-hRPE65v1 when administered to (b) (4) dogs via single subretinal or intravitreal injection	
<b>Study Animals</b>	<b>Breed</b>	(b) (4)
	<b>Species</b>	Dog
	<b>Age</b>	5-6 months old at dosing
	<b>Body Weight</b>	6.0 kg (males); 7.4 kg (females)
	<b>#/sex</b>	2M/4F
<b>Total #</b>	6	
<b>Test Article(s)</b>	AAV2-hRPE65v1	
<b>Control Article(s)</b>	180 mM Sodium Chloride/10 mM Sodium Phosphate, pH 7.3; (b) (4)	
<b>Route of Administration</b>	Subretinal or intravitreal injection	
<b>Study Groups and Dose Levels</b>	1.5 x 10 <sup>12</sup> vg/eye; see the table below (reproduced from the study report) for the study groups	
<b>Dosing Regimen</b>	Single administration (one or both eyes)	
<b>Randomization</b>	No	
<b>Description of Masking</b>	Not provided	
<b>Scheduled Sacrifice Time Point</b>	Day 24	

**Note:** Studies #9 and #10 were two separate studies with similar designs. The major differences is the study duration: approximately 3 weeks for Study #9 and 3 months for Study #10. The BD data are summarized for both Study #9 (3-week time point) and Study #10 (3-month time point) under Study #9.

### Comment:

- The expression of RPE65 from the test article, AAV2-hRPE65v1, is lower than AAV2-hRPE65v2 (see Study #3 in this review memo for these data). Thus, any potential toxicity due to the RPE65 protein cannot be adequately assessed using this vector construct. However, the BD profile of the AAV2 capsid and potential safety issues related to the AAV capsid can still be assessed using AAV2-hRPE65v1.

Group	Dose Level (vg)	Volume ( $\mu$ l)	Route (right eye)	Route (left eye)	Number of Animals	
					Male	Female
1. Vehicle – Both Eyes	0	150	Intravitreal Vehicle	Subretinal Vehicle	0	1
2. Test Article – One eye, High dose (100X); Vehicle – One Eye	$1.5 \times 10^{12}$	150	Subretinal Vehicle	Subretinal AAV	0	1
3. High dose (100X) (Subretinal)	$1.5 \times 10^{12}$	150	Uninjected	Subretinal AAV	2	1
4. High dose (100X) (Intravitreal)	$1.5 \times 10^{12}$	150	Uninjected	Intravitreal AAV	0	1

**Source:** Study Report #0406DC93.001, submitted in Module 4.2.3.1 of the BLA

### *Key Evaluations and Assessments:*

#### In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Twice daily
- Food consumption (quantitative): Once daily
- BWs: Prior to dosing on day 1, and once/week thereafter
- Ophthalmic examinations and intraocular pressure (IOP): Prior to dosing (baseline) and on days 2, 4, 8, 15, and 23
- Clinical pathology (hematology, chemistry): Prior to dosing and on days 8, 15, and 23
- ELISA for antibody response to RPE65 protein and AAV2 capsid (serum): Prior to dosing and on days 15 and 21
- ELISPOT assay for cytokine response (IFN $\gamma$  and IL-10) in PBMCs to AAV2 capsid and RPE65 protein: Prior to dosing and prior to sacrifice on day 24

#### Terminal (Day 24):

- Gross pathology
- Organ weights: Brain, heart, kidneys, liver, testes, ovaries, pancreas, preauricular lymph nodes, spleen, thyroids/parathyroids
- Systemic histopathology: Heart, kidneys, ovaries, testis, jejunum, colon, pancreas, liver, lung, bone marrow, skeletal muscle (thigh), brain, spleen, lymph nodes (preauricular), gross lesions
- Ocular histopathology: Injected and contralateral (uninjected) eyes, optic nerve
- BD: Pancreas, liver, lung, bone marrow, kidney, ovaries, testes, skeletal muscle (thigh), brain, spleen, lymph nodes (preauricular), optic chiasm, ocular anterior chamber fluid and ocular vitreous fluid (injected and contralateral), blood

#### *Key Results:*

- There were no unscheduled deaths.
- There were no test article related abnormal clinical observations, BWs, food consumption, clinical pathology, gross pathology, or organ weight findings.
- Ophthalmic examinations: On day 2, alterations in the appearance of the retinal fundus including retinal elevation, retinal de-attachment, retinal hemorrhage, and scar, were

observed in all animals. Per the veterinary ophthalmologist, these findings were attributed to the injection procedure. These lesions were resolving by day 4, in all dogs, with continued resolution on day 23.

- IOP: The IOP appeared to decrease (20-45%) in injected eyes (vector and control) compared to baseline and to uninjected eyes on days 2, 4, and 8. The days 15 and 23 IOP levels were comparable between injected and uninjected eyes.
- ELISA (serum): No specific antibody response to the human RPE65 transgene product was detected in any animal on days 15 and 23. A total of 3 of 6 vector-injected animals (1/1 in Group 2, 1/3 in Group 3, and 1/1 in Group 4) developed an antibody response, with a titer of 1:1000, to the AAV2 capsid (day 23).
- ELISPOT analysis: No detectable T cell response was detected on day 24 in any animal.
- Systemic histopathology (Day 24): Perivascular lymphocytic cuffing (minimal severity) was noted in the brain (brainstem and midbrain) of 1/3 Group 3 dogs. Perivascular infiltration of lymphoid cells (minimal severity) was observed in the choroid plexus of another Group 3 dog. Mixed perivascular infiltrates of inflammatory cells (minimal severity) were seen in the choroid plexus of the Group 4 animal. Per the veterinary pathologist, these findings were unique to the test article groups and thus considered test article related. The pathologist also stated that the presence of perivascular inflammatory cells suggested an immune response to the viral vector or an immune complex circulation.

**Comment:**

- The perivascular infiltration observed in the brain at 3 weeks was not observed at 3 months post-injection (see Study #10 in this review memo for details).
- Ocular histopathology (Day 24): The left eye of all Groups 2-4 animals injected (subretinal or intravitreal) with AAV2-hRPE65v1 had inflammatory lesions and RPE abnormalities.
  - Inflammation: Inflammatory lesions were present in the inner retina and posterior chamber. Perivascular inflammation was present in the nerve fiber and ganglion cell layers. The severity varied from mild (only a few inflammatory cells surrounding the vessel) to severe. Inflammatory cells consisted predominantly of macrophages and lymphocytes, with fewer plasma cells, accompanied by reactive endothelial hypertrophy. Per the pathologist, this pattern is consistent with activation of the adaptive immune system.
  - RPE abnormalities: RPE lesions were limited to the central retina. RPE lesions consisted of multifocal flattening and loss of epithelium, accompanied by scattered hypertrophy and pigment clumping of RPE cells. The lesions were accompanied by accumulation of small numbers of microglia, pigment-laden macrophages, and

occasional PR cell nuclei in the subretinal space. Per the pathologists, these lesions are consistent with RPE cell death, followed by regenerative effects.

- **BD:**
  - There was no evidence of vector presence in the testis/ovary, pancreas, liver, lung, bone marrow, spleen, kidney, preauricular lymph nodes, brain, or skeletal muscle at 3 weeks or 3 months post-injection.
  - Intra-ocular fluids (anterior chamber and vitreous) of all test article-injected eyes were strongly positive (up to  $10^6$  copies/ $\mu\text{g}$  gDNA) for vector presence at 3 weeks and 3 months post-injection. There was no significant difference in DNA copies in fluids collected from eyes injected subretinally or intravitreally at either time point. The intraocular fluids collected from control eyes were negative.
  - The optic nerve (with optic chiasm) of the test article-injected eyes was weakly positive (up to 600 copies/ $\mu\text{g}$  gDNA) for vector presence at 3 weeks and 3 months.
  - The pre-auricular lymph nodes, collected at 3 months from two animals, were positive (Dog #5 with 4024 copies/ $\mu\text{g}$  gDNA and Dog #12 at  $<100$  copies/ $\mu\text{g}$  gDNA).

**Comment:**

- Per the study report, the positive read-out for the pre-auricular lymph nodes was due to migration of macrophages (that engulfed test article-containing cells) to this draining lymph node. A very low level of vector DNA ( $<50$  copies/ $\mu\text{g}$  gDNA) was also detected in pre-auricular lymph nodes in the NHPs at 3 months post-subretinal injection in Study #11.

**Summary from the study report:**

- No systemic toxicity was observed.
- There were no definitive vector-related histopathological findings in the non-ocular tissues examined, except for mild and transient inflammation identified in the brain.
- Following a single vector administration, normal-sighted dogs developed antibodies to the AAV2 capsid (detected in the serum).
- Ocular histopathological evaluations of vector-injected eyes showed test article-related inflammatory lesions and pathology of the RPE for all the test article-injected eyes. However, the dose level of  $1.5 \times 10^{12}$  vg/eye is 10-fold higher than the dose level of  $1.5 \times 10^{11}$  vg/eye in the 'Dosage and Indication' section of the label.
- The BD data show the absence of vector in the non-ocular tissues, including gonads.

**Study #10:**

<b>Report Number</b>	0470DC93.001	
<b>Date Report Signed</b>	June 25, 2009	
<b>Title</b>	Three-Month Toxicity Study of AAV2/2.CBA.hRPE65 Following a Single Subretinal or Intravitreal Injection in (b) (4) Dogs	
<b>GLP Status</b>	Yes	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the toxicity of AAV2-hRPE65v1 when administered to (b) (4) dogs via single subretinal or intravitreal injection	
<b>Study Animals</b>	<b>Breed</b>	(b) (4)
	<b>Species</b>	Dog
	<b>Age</b>	6-7 months
	<b>Body Weight</b>	5.7-7.7 kg
	<b>#/sex</b>	2M/4F
	<b>Total #</b>	6
<b>Test Article(s)</b>	AAV2-hRPE65v1	
<b>Control Article(s)</b>	180mM Sodium Chloride/10mM Sodium Phosphate pH 7.3, (b) (4)	
<b>Route of Administration</b>	Subretinal or intravitreal injection	
<b>Study Groups and Dose Levels</b>	1.5 x 10 <sup>12</sup> vg/eye; see the table below (reproduced from the study report) for the study groups	
<b>Dosing Regimen</b>	Single administration (one or both eyes)	
<b>Randomization</b>	No	
<b>Description of Masking</b>	Not provided	
<b>Scheduled Sacrifice Time Point</b>	Day 92	

Group	Dose Level (vg)	Volume (µl)	Route (right eye)	Route (left eye)	Number of Animals	
					Male	Female
1. Vehicle – Both Eyes	0	150	Intravitreal Vehicle	Subretinal Vehicle	0	1
2. Test Article – One eye, High dose (100X); Vehicle – One Eye	1.5x10 <sup>12</sup>	150	Subretinal Vehicle	Subretinal AAV	0	1
3. High dose – 100X (Subretinal)	1.5x10 <sup>12</sup>	150	Uninjected	Subretinal AAV	2	1
4. High dose – 100X (Intravitreal)	1.5x10 <sup>12</sup>	150	Uninjected	Intravitreal AAV	0	1

**Source:** Study Report #0470DC93.001, submitted in Module 4.2.3.1 of the BLA

**Key Evaluations and Assessments:****In-life:**

- Mortality/morbidity: Twice daily
- Clinical observations: Twice daily
- Food consumption (quantitative): Once daily
- BWs: Prior to dosing on day 1, and once/week thereafter
- Ophthalmic examinations and IOP: Prior to dosing (baseline) and on days 2, 8, 15, 28, 56, and 88
- Clinical pathology (hematology, chemistry): Prior to dosing and on days 8, 15, 28, 56, and 92

- ELISA for antibody response to RPE65 protein and AAV2 capsid (serum): Prior to dosing and on days 15, 28, and 92
- ELISPOT assay for cytokine response (IFN $\gamma$  and IL-10) in PBMCs to AAV2 capsid and RPE65 protein: Prior to dosing and prior to sacrifice on day 92

Terminal (Day 92):

- Gross pathology
- Organ weights: Brain, heart, kidneys, liver, testes, ovaries, pancreas, preauricular lymph nodes, spleen, thyroids/parathyroids
- Systemic histopathology: Heart, kidneys, ovaries, testis, jejunum, colon, pancreas, liver, lung, bone marrow, skeletal muscle (thigh), brain, spleen, lymph nodes (preauricular), gross lesions
- Ocular histopathology: Injected and contralateral (uninjected) eyes, with optic nerve
- BD: Pancreas, liver, lung, bone marrow, kidney, ovaries, testes, skeletal muscle (thigh), brain, spleen, lymph nodes (preauricular), optic chiasm, ocular anterior chamber fluid and ocular vitreous fluid (injected and contralateral), blood

*Key Results:*

- There were no unscheduled deaths.
- There were no test article related abnormal clinical observations, BWs, food consumption, clinical pathology, gross pathology, organ weights, or systemic histopathology findings.
- Ophthalmic examinations:
  - Retinal fundus lesions (e.g., retinal elevation, retinal de-attachment, retinal hemorrhage, scar), which (per the veterinary ophthalmologist) appeared to be the result of the injection procedure, were observed on day 2 in most of the injected eyes (vehicle and test article). These lesions appeared to be resolving on days 8, 15, 28, and 56. No lesion was apparent in any examined eye on day 88.
  - On day 88, hyper-reflectivity of the tapetal region, which (per the veterinary ophthalmologist) appeared to represent focal to multifocal geographic areas of retinal degeneration surrounding the area of the original injection, was observed in all injected eyes for Groups 2 and 3 animals.  
**Note:** Retinal degeneration was also observed microscopically.
- IOP: The IOP levels on day 2 decreased in the injected eyes compared to respective baseline (Groups 1-4) and to uninjected eyes (Groups 3-4). The IOP levels were comparable to baseline levels on days 8, 28, 56, and 88 for all groups.
- ELISA (serum):
  - No specific antibody response to the human RPE65 transgene product was detected in any animal.

- A total of 4 of 6 vector-injected animals developed antibodies to the AAV2 capsid, with titer of 1:1000 in one Group 2 animal and titers of 10,000 in the remaining three animals (one each in Groups 2-4).
- ELISPOT analysis: There was no detectable T cell response in animals subretinally or intravitreally injected with AAV2-hRPE65v1.
- Ocular histopathology (Day 92):
  - There were no major lesions in the right eyes of any Group 1-4 animals (vehicle injected or uninjected). Inflammatory lesions were noted in all left eyes injected with AAV2-hRPE65v1 (subretinal or intravitreal), but not in the Group 1 vehicle control-injected left eye. Inflammation was observed in the choroid, tapetum, and subretinal space and appeared directed towards the RPE. Pathology of the outer nuclear layer in the inflamed regions ranged from: 1) loss of outer segments, 2) periodic pseudo-rosette formation, and 3) complete loss of the outer nuclear layer, with retinal detachment and partial loss of the inner nuclear layer.
  - Perivascular inflammatory lesions similar to those observed in the 3-week dog study (Study #9 in this review memo) were noted at a greater severity in this study, and did not resolve by the 3-month time point. The lesions often lead to focal retinal degeneration. According to the pathologist, this finding was due to a severe adaptive immune response directed towards the RPE.
- BD: See Study #9 for this information.

**Comment:**

- Per the applicant, since RPE65 transgene expression with AAV2-hRPE65v1 is lower compared to AAV2-hRPE65v2 (see Study #3 for details), the observed ocular toxicities in Studies #9 and #10 were related to the high dose of AAV2 vector administered and were not due to expression of the RPE65 protein. This reviewer agrees with this explanation.

**Summary from the study report:**

- No systemic toxicity was observed.
- Following a single vector administration, normal-sighted dogs developed antibodies to the AAV2 capsid (detected in the serum).
- There were no definitive vector-related histopathological findings noted in the non-ocular tissues examined.
- Inflammatory lesions were present in all eyes injected with AAV2-hRPE65v1. The ocular perivascular inflammation observed microscopically at 3 weeks (Study #9), was also present at 3 months, but was more severe.

**Study #11:**

<b>Report Number</b>	XHB00009										
<b>Date Report Signed</b>	December 12, 2008										
<b>Title</b>	Three-Month Toxicity Study of AAV2/2.CBA.hRPE65v2 Following a Single Subretinal Injection in (b) (4) Monkeys										
<b>GLP Status</b>	No										
<b>Testing Facility</b>	(b) (4)										
<b>Objective(s)</b>	To determine the toxicity of AAV2-hRPE65v2 when administered to (b) (4) monkeys via a single subretinal injection										
	<table border="1"> <tr> <td><b>Species</b></td> <td>(b) (4) monkey</td> </tr> <tr> <td><b>Age</b></td> <td>2-3 years old</td> </tr> <tr> <td><b>Body Weight</b></td> <td>2.1-2.5kg</td> </tr> <tr> <td><b>#/sex</b></td> <td>1M/4F</td> </tr> <tr> <td><b>Total #</b></td> <td>5</td> </tr> </table>	<b>Species</b>	(b) (4) monkey	<b>Age</b>	2-3 years old	<b>Body Weight</b>	2.1-2.5kg	<b>#/sex</b>	1M/4F	<b>Total #</b>	5
<b>Species</b>	(b) (4) monkey										
<b>Age</b>	2-3 years old										
<b>Body Weight</b>	2.1-2.5kg										
<b>#/sex</b>	1M/4F										
<b>Total #</b>	5										
<b>Test Article(s)</b>	AAV2-hRPE65v2										
<b>Control Article(s)</b>	PBS - 0.001% (b) (4) (see the Comment below)										
<b>Route of Administration</b>	Subretinal injection										
<b>Study Groups and Dose Levels</b>	See the table below (reproduced from the study report) for the study groups										
<b>Dosing Regimen</b>	Single administration (one or both eyes)										
<b>Randomization</b>	No										
<b>Description of Masking</b>	The pathologist was masked as to which eye received vehicle or AAV2-hRPE65v2										
<b>Scheduled Sacrifice Time Point</b>	Day 92										

**Comment:**

- Per the applicant, both the vehicle and vector formulation contained 0.001% Pluronic (b) (4), which is (b) (4) concentration in the GLP single dose toxicology studies in dogs (Studies #9 and 10 in this review memo). Device compatibility studies demonstrated that use of this surfactant concentration resulted in more consistent and quantitative recovery of AAV vector following passing through the subretinal delivery device. However, there was no significant loss of vector formulated at the lower concentration of surfactant following passing through the delivery device.

Gr. No.	Number of Animals	Test Material	Vector Dose (vg/eye)		Dose Volume (µL/eye)		Dosing Day
			Right Eye	Left Eye	Right Eye	Left Eye	
1	1	Vehicle (right eye) AAV2/2.CBA.hRPE65v2 (left eye)	0	$7.5 \times 10^{11}$	150	150	Day 1
2	1	Vehicle (left eye) AAV2/2.CBA.hRPE65v2 (right eye)	$7.5 \times 10^{11}$	0	150	150	
3	1	AAV2/2.CBA.hRPE65v2 (both eyes)	$7.5 \times 10^{11}$	$7.5 \times 10^{11}$	150	150	
4	1		$3.0 \times 10^{11}$	$3.0 \times 10^{11}$	150	150	
5	1		$3.0 \times 10^{11}$	$3.0 \times 10^{11}$	150	150	

Gr. = group, vg = vector genome.

**Source:** Study Report #XHB-00009, submitted in Module 4.2.3.1 of the BLA

*Key Evaluations and Assessments:*In-life:

- Mortality/morbidity: Twice daily
- Clinical observations: Twice daily
- BWs: Twice prior to dosing (days -3 and 1), and once weekly thereafter
- Food consumption (quantitative): Once daily
- Ophthalmic examinations: Days -1, 3, 28, 56, 88, and 92
- ELISA for antibody response to RPE65 protein and AAV2 capsid (serum): Prior to dosing (day 1) and on days 21 and 92
- ELISPOT assay for inflammatory cytokine response (IFN $\gamma$ ) in PBMCs to AAV2 capsid and RPE65 protein: Prior to dosing (day 1) and on days 21 and 92

Terminal (Day 92):

- Gross pathology
- Systemic histopathology: Brain, intestine, colon, skeletal muscle, skin, liver, kidney, lung
- Ocular histopathology: Injected and contralateral (uninjected) eyes, optic chiasm
- BD: Aqueous humor (right and left), vitreous humor (right and left), optic nerve (right and left), skin, skeletal muscle, sciatic nerve, bone marrow, lymph node, colon, duodenum, stomach, urinary bladder, ovaries, uterus, cervix, vagina, pancreas, spleen, adrenal gland, kidney, liver, gallbladder, diaphragm, bone, thymus, heart, lung, thyroid, trachea, esophagus, aorta, spinal cord, brain, optic chiasm, salivary gland

*Key Results:*

- There were no unscheduled deaths.
- There were no test article related abnormal clinical observations, BWs, food consumption, gross pathology, or systemic histopathology findings.
- Ophthalmic examinations: Similar acute and chronic findings were noted in both vehicle-injected and vector-injected eyes, deemed (by the veterinary ophthalmologist) likely related to the surgery procedure. Acute findings consisted of conjunctival hyperemia, vitreal hemorrhage and fibrin, and retinal changes (detachment, hemorrhage, and pigment changes). Changes observed beyond day 28 included retinal scarring, with pigment changes and detachments.
- ELISA (serum): No specific antibody response to the human RPE65 transgene product or to the AAV2 capsid was detected in any animal.
- ELISPOT analysis: There was no detectable cell response (measured by secretion of IFN $\gamma$ ) directed to the AAV2 capsid in PBMCs.
- Ocular histopathology:
  - Per the pathologist, the following findings observed at the subretinal injection site in vehicle-injected and vector-injected eyes, were most likely due to the surgical procedure: occasional hypertrophy and clumping of RPE cells, focal loss of PR cells,

- a few pigment-laden macrophages, occasional subtle RPE abnormalities (including loss of pigment granules), and a few detached RPE cells.
- Mild inflammation was present in focal spots either perivascular or at the optic nerve head in two eyes injected with the  $3 \times 10^{11}$  vg/eye. Per the pathologist, since there was no clear dose response effect with respect to these changes, possible attribution to the surgical procedure was made.
  - BD:
    - There was no evidence of vector spread to pancreas, lung, bone marrow, kidney, testes/ovaries, brain, diaphragm, systemic lymph nodes, bone, thymus, heart, urinary bladder, stomach, colon, skeletal muscle, or skin for any animal.
    - The intra-ocular fluids (anterior chamber fluid and vitreous) of all vector-injected eyes were strongly positive (up to  $10^7$  copies/ $\mu$ g gDNA).
    - The optic nerves (and optic chiasms) of all vector-injected eyes were weakly positive (up to 1571 copies/ $\mu$ g gDNA).
    - The vector was detected in the spleen and liver in a dose-dependent manner. At the high-dose ( $7.5 \times 10^{11}$  vg/eye), samples from the spleen (up to 1539 copies/ $\mu$ g gDNA) and liver (up to 415 copies/ $\mu$ g gDNA) were positive in all vector-injected animals, with weak (up to 244 copies/ $\mu$ g gDNA in spleen and 61 copies/ $\mu$ g gDNA in liver) or no signal identified at the low-dose ( $3 \times 10^{11}$  vg/eye).
    - A very low level of vector DNA (<50 copies/ $\mu$ g gDNA) was detected in pre-auricular lymph nodes in two high-dose NHPs.

#### **Summary from the study report:**

- After a single subretinal injection of AAV2-hRPE65v2 in NHPs, there were no test article related systemic toxicities. No humoral or cellular immune response to the AAV2 capsid or RPE65 transgene product was detected.
- Ophthalmic examination and ocular histopathology findings were most likely related to the surgery procedure.
- Following single administration, healthy NHPs did not develop antibodies to the AAV2 vector capsid.
- Vector presence in non-ocular tissues was observed in a dose-dependent manner, with weak or no signal detected at  $3 \times 10^{11}$  vg/eye.
- Based on these findings, the NOAEL is determined as  $7.5 \times 10^{11}$  vg/eye in NHPs, which is 5-fold higher than the than the dose level of  $1.5 \times 10^{11}$  vg/eye in the 'Dosage and Indication' section of the proposed label.

**Study #12:**

<b>Report Number</b>	(b) (4) NHP-5	
<b>Date Report Signed</b>	June 27, 2012	
<b>Study Title</b>	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 in Non-Human Primates	
<b>GLP Status</b>	No	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the safety of re-administration of AAV2-hRPE65v2 when administered to NHPs via subretinal injection. To determine the safety of subretinal administration of AAV2-hRPE65v2 in NHPs that had previous exposure to AAV vectors	
<b>Study Animals</b>	<b>Species</b>	(b) (4) monkeys
	<b>Age</b>	9-13 years old
	<b>Body Weight</b>	3.5-9.5 kg
	<b>#/sex</b>	4 females (2/species)
	<b>Total #</b>	4
<b>Test Article(s)</b>	AAV2-hRPE65v2	
<b>Control Article(s)</b>	N/A	
<b>Route of Administration</b>	Subretinal injection	
<b>Study Groups and Dose Levels</b>	1.5 x 10 <sup>11</sup> vg/eye; see Table 1 below (reproduced from the study report) for the study groups	
<b>Dosing Regimen</b>	Days 0 (right eye) and 51 (contralateral left eye)	
<b>Randomization</b>	No	
<b>Description of Masking</b>	The pathologist was masked as to the order of the eyes which received AAV2-hRPE65v2	
<b>Scheduled Sacrifice Time Points</b>	Days 190 (two animals) and 217 (two animals)	

Table 1: Dose Levels and Schedule

Animal/ID	Dose Level (Subretinal) in Right Eye, 12/23/08 (Day 0)	Dose Level (Subretinal) in Left Eye, 2/12/09 (Day 51)
	(vg)	(vg)
1. 99E126 ((b) (4) female)	1.5X10 <sup>11</sup>	1.5X10 <sup>11</sup>
2. 99E146 ((b) (4) female)	1.5X10 <sup>11</sup>	1.5X10 <sup>11</sup>
3. AJ75 ((b) (4) female; (b) (4))	1.5X10 <sup>11</sup>	1.5X10 <sup>11</sup>
4. AP9X ((b) (4) female; (b) (4))	1.5X10 <sup>11</sup>	1.5X10 <sup>11</sup>

**Source:** Study Report #((b) (4)) NHP-5, submitted in Module 4.2.3.2 of the BLA

**Note:** Per the study report, the NHPs were administered a variety of serotypes of AAV vectors systemically via the respiratory (intranasal), intravenous, or intramuscular route. Thus, the animals were not naïve to AAV vectors, and NAbs to AAV2 were detected in all four NHPs upon screening.

**Key Evaluations and Assessments:****In-life:**

- Mortality/morbidity: Twice daily

- Clinical observations and appetite: Once daily
- BWs: Day 0, two months after the first injection, and prior to sacrifice
- Ophthalmic examinations: Prior to injection, on days 3, 7, 14 post-injection, and prior to sacrifice
- ELISA for antibody analysis (anti-AAV2 capsid NABs in serum and anterior chamber fluid): see Table 2 below (reproduced from the study report)
- ELISA for antibody analysis (anti-RPE65 antibodies in serum and anterior chamber fluid): see Table 2 below
- T cell response to the AAV2 capsid and the RPE65 transgene product by IFN $\gamma$  ELISPOT analysis of PBMCs: see Table 2 below

Table 2: Specimen Collection Schedule

Animal/ID	11/14/08	12/22/08	12/23/08	2/12/09	3/6/09	7/1/09 Termination Cyno	7/20/09	7/27/09 &7/28/09 Termination Rhesus
	Screening	Day -1	Day 0	Day 51	Day 73	Day 190	Day 210	Day 217
1. 99E126 (b) (4) female	Serum	Serum PBMC	Right AC fluid	Serum Left & Right AC fluid PBMC	Serum PBMC	Serum	Serum PBMC	Serum Left & right AC fluid PBMC
2. 99E146 (b) (4) female	Serum	Serum PBMC	Right AC fluid	Serum Left & Right AC fluid PBMC	Serum PBMC	Serum	Serum PBMC	Serum Left & right AC fluid PBMC
3. AJ75 (b) (4) female; (b) (4) (b) (4)	Serum	Serum PBMC	Right AC fluid	Serum Left & Right AC fluid	Serum PBMC	Serum Left & right AC fluid PBMC	NA	NA
4. AP9X (b) (4) female; (b) (4) (b) (4)	Serum	Serum PBMC	Right AC fluid	Serum Left & Right AC fluid PBMC	Serum PBMC	Serum Left & right AC fluid PBMC	NA	NA

**Source:** Study Report #(b) (4) NHP-5, submitted in Module 4.2.3.2 of the BLA

#### Terminal (Days 190 and 217):

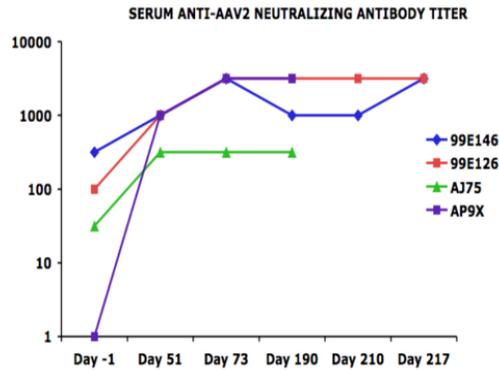
- Gross pathology
- Ocular histopathology: eyes, optic nerve, and gross lesions
- IHC: RPE65 protein presence in the eyes

#### *Key Results:*

- There were no unscheduled deaths.
- There were no test article related abnormal clinical observations, BWs, appetite, or gross pathology findings.
- Ophthalmic examinations: Mild and reversible inflammatory responses were observed in all eyes following injection. At the last ophthalmic examination (prior to sacrifice), for all eyes, there was no abnormal findings observed in the retina, and the ocular media (aqueous, lens, and vitreous) was clear.

- Antibody analysis (anti-AAV2 capsid Nabs):
  - Serum: NAbs to the AAV2 capsid were detected in the serum in all animals at baseline (day 0). The antibody titers increased after administration of AAV2-hRPE65v2 (day 51) and remained elevated for the duration of the study (Figure 8; reproduced from the study report).

Figure 8. Serum neutralizing antibody titer to the AAV2 vector capsid.



Source: Study Report # (b) (4) NHP-5, submitted in Module 4.2.3.2 of the BLA

- Anterior chamber fluids:
  - Right eye (first injected eye): On day 0, NAbs to the AAV2 capsid were not detected in 3/4 animals and marginally positive in one animal (#99E146), that also had the highest serum NAb titer (1:1000-1:3000) at baseline. NAbs were detected in all four eyes at day 51.
  - Left eye (second injected eye): On day 51, NAbs to the AAV2 capsid were not detected in 2/4 animals and a low titer was present in 2/4 animals. The titers were increased at the time of sacrifice.
  - Right and left eyes: Nab titers remained elevated for the duration of the study; however, the titers were at levels lower than those in the serum.
- Antibody analysis (anti-RPE65 antibody):
  - Serum: No titers to human RPE65 were detectable at any time point, except for one animal (#99E126) that had a serum titer of 1:1000 at all time points tested, including baseline.
  - Anterior chamber fluid: No titers to human RPE65 were detected at any time point tested.
- T cell response to the AAV2 vector capsid and the RPE65 transgene product (in PBMCs):
  - No T cell response to the RPE65 transgene product was detected in any animal at any time point.

- No T cell response to the AAV2 capsid was detected at baseline (day 0). A T cell response to the AAV2 vector capsid was detected in 1/4 animals (#99E126) at days 51, 73, 210, and 217. Further characterization of the T cell response in this animal showed that it was mediated by CD4+ T cells, but not cytotoxic CD8+ T cells.
- Animal #AJ75 had a borderline positive T cell response to the AAV capsid at day 190.
- Ocular histopathology: Focal ocular lesions were observed in both eyes of all four animals, including:
  - Occasional inflammatory cells (lymphocytes) were identified in the vitreous, and rare inflammatory cells were found in the subretinal space, retina, and optic nerve. Per the pathologist, these findings indicated that there may be a mild immune response due to administration of AAV2-hREP65v2 to the contralateral eye. The pathologist also noted that there was no apparent association of the inflammatory findings with the order in which the eyes were injected or with the pre-existing immune response to AAV2.
  - Some red blood cells were noted in the vitreous.
  - There was focal scarring at the retinotomy site.
  - There was no degeneration of the neural retina.
- IHC: Increased levels of RPE65 protein were detected only in the RPE within the subretinal injection site. There was no evidence of RPE65 protein in the neural retina or optic nerve.

**Summary from the study report:**

- Sequential administration of AAV2-hRPE65v2 in the contralateral eye approximately two months after administration into the first-eye did not result in significant adverse ocular effects, even in the presence of an existing humoral response to the AAV2 capsid.
- Following sequential administration into the eyes of NHPs previously exposed to AAV2, antibody titers to the AAV2 capsid increased and remained elevated for the duration of the study.
- There appeared to be a mild immune response upon administration of AAV2-hRPE65v2 in the contralateral eye.

**Study #13:**

**Note:** Study #13 evaluated a dosing paradigm that is not directly applicable to the dosing regimen for LUXTURNA™ specified in the ‘Dosage and Indication’ section of the proposed label (i.e., bilateral, sequential).

<b>Report Number</b>	(b) (4) Canine Unaffected-4	
<b>Date Report Signed</b>	June 27, 2012	
<b>Study Title</b>	Safety of Subretinal Re-Administration of AAV2-hRPE65v2 in Unaffected Canines	
<b>GLP Status</b>	No	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the safety of re-administration of AAV2-hRPE65v2 when administered to unaffected dogs via subretinal injection	
<b>Study Animals</b>	<b>Breed</b>	N/A; dogs were carriers for lysosomal storage disease
	<b>Species</b>	Dog
	<b>Age</b>	13-14 months old
	<b>Body Weight</b>	5-8 kg
	<b>#/sex</b>	1M/2F
	<b>Total #</b>	3
<b>Test Article(s)</b>	AAV2-hRPE65v2	
<b>Control Article(s)</b>	N/A	
<b>Route of Administration</b>	Subretinal injection	
<b>Study Groups and Dose Levels</b>	1.5 x 10 <sup>11</sup> vg/eye/injection; see Table 1 below (reproduced from the study report)	
<b>Dosing Regimen</b>	Days 0 (left eye), 98 (contralateral right eye), and 140 (right eye)	
<b>Randomization</b>	No	
<b>Description of Masking</b>	The pathologist was masked as to the order of the eye that received AAV2-hRPE65v2.	
<b>Scheduled Sacrifice Time Point</b>	Day 196 (6.5 months post-first injection)	

**Table 1. Subretinal Injection Dates for Bilateral Re-Administration Study in Un-Affected Dogs**

Animal ID	Left Eye	Right Eye	Right Eye	Cumulative Dose
K332 (black female; cage card 0662151)	Subretinal 1.5E11 vg (7/24/07; Day 0)	Subretinal 1.5E11 vg (10/30/07; Day 98)	Surgery; no injection (12/11/07; Day 140)	3.0E11 vg
K333 (grey female; cage card 0662150)	Subretinal 1.5E11 vg (7/24/07; Day 0)	Subretinal 1.5E11 vg (10/30/07; Day 98)	Subretinal 1.5E11 vg (12/11/07; Day 140)	4.5E11 vg
K334 (male; cage card 0662149)	Subretinal 1.5E11 vg (7/24/07; Day 0)	Subretinal 1.5E11 vg (10/30/07; Day 98)	Subretinal 1.5E11 vg (12/11/07; Day 140)	4.5E11 vg

**Source:** Study Report #(b) (4) Canine Unaffected-4, submitted in Module 4.2.3.2 of the BLA

**Key Evaluations and Assessments:****In-life:**

- Mortality/morbidity: Twice daily
- Clinical observations and appetite: Once daily
- BWs: Prior to first injection and occasional intervals thereafter

- Ophthalmic examinations: Prior to first injection, days 3, 7, 14,15, 28-43, and 6 months post-first injection
- Specific ophthalmic examinations for inflammation and any flattening of the retina due to detachment: Days 2, 3, 13-15, 98, and 140 post-first injection (left eye); Days 1-3, 42, and 71 post-second injection (right eye); Days 1-3 and 29 post the third injection (right eye)
- Antibody analysis for AAV2 capsid NAbs (serum): Day 196
- ELISA for anti-AAV2 capsid antibodies (anterior chamber fluid): Baseline and after each injection
- ELISPOT (IFN $\gamma$  and IL-10) assay for T cell response to the AAV2 capsid and the RPE65 transgene product in PBMCs: Days 140 and 196

Terminal (Day 196):

- Selective gross pathology – Eyes, optic nerve, and optic chiasms
- Ocular histopathology – Eyes, optic nerve, and optic chiasms
- Immunofluorescence to detect the presence and location of RPE65 protein in the eyes

*Key Results:*

- There were no adverse effects on clinical observations, BWs, and appetite.
- Ophthalmic examinations:
  - Animal #K332:
    - Hyphema developed in the right eye on day 98 following the second injection and resolved spontaneously within two days.
    - A choroidal hemorrhage and “8-ball hyphema” developed in the right eye on day 140 while performing the second injection. The subretinal injection was halted due to concerns that this surgical complication would result in sympathetic ophthalmia. The dog’s IOP was normal and the hemorrhage resolved after two weeks.
    - Left eye corneal irritation developed on day 175 and was responsive to two days of topical steroids/antibiotics.

**Comment:**

- Per the study report, while it cannot be completely excluded that the corneal changes in the left eye of #K332 on day 175 were caused by an immune response following repeat injection of AAV2-hRPE65v2, the possibility is remote. The Study Director stated that given that the inflammation occurred one month after the choroidal hemorrhage in the right eye and there was no evidence of corneal infiltrates by histopathologic examination, the most likely explanation for the inflammation is sympathetic ophthalmia resulting from the surgical complication to the right eye on day 140.
- Animal #K333: There were no signs of inflammation in the sclera, conjunctiva, cornea, or retina at any time point.

- Animal #K334: Hyphema developed in the right eye on day 140 following the second injection and resolved spontaneously within two days. There were no other abnormal findings.
- Antibody analysis:
  - Serum: Anti-AAV2 capsid Nabs (1:316-1:1000) were detected in all animals at day 196.
  - Anterior chamber fluid: Anti-AAV2 capsid antibodies were detected in each eye following its injection (titer of 1:100-1:1000). The highest antibody titers (1000-10000) were found at day 196 in the right eyes of Animals #K333 and #K334. These dogs were injected in this eye on days 98 and 140 (Table 1; reproduced from the study report).

Table 1. Anti-AAV2 antibody titers in intra-ocular fluid obtained at pre-injection and post-injection timepoints. Note: The right eye of K332 was not re-injected.

ND, non-detectable; NA, not available.

	Baseline: Pre- Injection Left	Post injection left; Pre- Injection Right	Post bilateral injection; Pre Re-Injection Right	Terminal
Date	7/24/07	10/30/07	12/11/07	2/5/08
K332: Right	NA	ND	100-1,000	100-1,000
K332: Left	NA	ND	NA	100-1,000
K333: Right	NA	NA	1,000-10,000	1,000-10,000
K333: Left	NA	100-1,000	NA	100-1,000
K334: Right	NA	NA	NA	1,000-10,000
K334: Left		100-1,000	NA	100-1000

**Source:** Study Report #(b) (4) Canine Unaffected-4, submitted in Module 4.2.3.2 of the BLA

- **G-W ratio:** Antibody titers to AAV2 in the day 196 anterior chamber fluid were compared to anti-AAV2 capsid Nab titers in the day 196 sera. The G-W ratio was 0.3 in the left eyes of #K333 and #K334, which was elevated to a ratio of 3 in the right eyes. The G-W ratio did not change between the left and right eye for #K332.  
**Note:** The G-W ratio was calculated using data generated from two different assays (ELISA vs. NAb assay) that were performed in two different laboratories.
- **T cell immune response (PBMCs):** The first or subsequent subretinal injections of AAV2-hRPE65v2 did not result in a Th1 (CD8+) T cell immune response to either the

AAV2 capsid or to the expressed hRPE65 protein. A positive T cell response to the hRPE65 transgene product was detected at day 140 using IL-10 ELISPOT assay.

**Note:** Detection of IL-10 is usually associated with immune tolerance rather than immunogenicity.

- RPE65 Immunofluorescence: Strong staining for RPE65 protein was only detected in the RPE of the normal-sighted eyes from healthy animals.

**Note:** The endogenous RPE65 protein cannot be distinguished from the human RPE65 protein derived from AAV2-hRPE65v2 administration. Levels of RPE65 immunofluorescence were similar in the eyes, except for reduced immunofluorescence in regions with scarring or extensive rosette formation.

- Ocular histopathology: See the table below:

Injected eye	Uninjected regions	Injected regions
<b>Single injection (left eye of all animals and right eye of #K332)</b>	Normal structure without apparent inflammatory cells	<ul style="list-style-type: none"> <li>• Focal regions of scarring and rosette formation</li> <li>• Occasional inflammatory cells including plasma cells and lymphocytes in the vitreous: in the subretinal space and overlying the optic nerve head</li> </ul>
<b>Two sequential injections (right eye of #K333 and #K334)</b>	Apparent inflammatory cells in vitreous	<ul style="list-style-type: none"> <li>• Occasional inner retinal blood vessels showing peri-vascular cuffing</li> <li>• Focal regions of scarring, invaginations of the photoreceptor layer, and rosette formation</li> <li>• Increased number of inflammatory cells in the vitreous and optic nerve head compared to eyes with a single injection</li> </ul>

#### Summary from the study report:

- There were no signs of systemic toxicity following repeat subretinal injection of AAV2-hRPE65v2.
- All animals had anti-AAV2 NAb antibodies in the serum. Anti-AAV antibodies were detected in the anterior chamber fluid following each injection.
- There was no evidence of a Th1 (CD8+) T cell response to either the AAV2 capsid or the human RPE65 protein in PBMCs.
- Ocular histopathology showed a mild immune response, which is possibly related to re-administration of AAV2-hRPE65v2 sequentially to the same eye. **Note:** Vector re-administration to the same eye is not specified for LUXTURNA™ in the ‘Dosage and Indication’ section of the proposed label (i.e., bilateral, sequential).

**Study #14:**

**Note:** Study #14 evaluated a dosing paradigm (i.e., bilateral, sequential or ipsilateral) that is not directly applicable to the dosing regimen for LUXTURN<sup>TM</sup> in the ‘Dosage and Indication’ section of the proposed label.

This toxicology study also evaluated activity endpoints following administration of AAV2-hRPE65v2.

<b>Report Number</b>	(b) (4) Canine Ipsilateral-3	
<b>Date Report Signed</b>	June 27, 2012	
<b>Study Title</b>	Safety of Ipsilateral Subretinal Re-Administration of AAV2-hRPE65v2 in Affected Canines	
<b>GLP Status</b>	No	
<b>Testing Facility</b>	(b) (4)	
<b>Objective(s)</b>	To determine the safety of re-administration of AAV2-hRPE65v2 when administered to affected (b) (4) dogs to the initially injected eye via subretinal injection	
<b>Study Animals</b>	<b>Breed</b>	(b) (4) (RPE65-/-)
	<b>Species</b>	Dog
	<b>Age</b>	Three dogs were 4 months old, one (Ripley) was 8.5 months old, and one (Venus) was 2.75 years old
	<b>Body Weight</b>	8-30 kg
	<b>#/sex</b>	2M/3F
	<b>Total #</b>	5
<b>Test Article(s)</b>	AAV2-hRPE65v2	
<b>Control Article(s)</b>	N/A	
<b>Route of Administration</b>	Subretinal injection	
<b>Study Groups and Dose Levels</b>	8.25 x 10 <sup>10</sup> - 1.1 x 10 <sup>11</sup> vg/eye; see Table 1 below (reproduced from the study report)	
<b>Dosing Regimen</b>	Bilateral, simultaneous/re-administration: days 0 (left and right eye) and 34 (left eye), except for Venus and Saturn (see Table 1 below) (reproduced from the study report)	
<b>Randomization</b>	No	
<b>Description of Masking</b>	ERGs and ocular histopathology examination	
<b>Scheduled Sacrifice Time Points</b>	Three dogs were sacrificed at 2.5 months post-first injection. Two dogs (Venus and Mercury) were adopted out as companion animals at 5-6 months after the last (third) injection.	

Table 1. Subretinal Injection Dates for Ipsilateral Re-Administration Study in Affected Dogs

Animal ID	Right Eye	Left Eye: {(1) injection and (2) re-injection}
Venus (female; DO6-295)	Subretinal (1.5E11 vg; 150 µl; 8/14/07; <u>previous study</u> )	1) Subretinal (1.5E11 vg; 150 µl; 8/28/07; <u>previous study</u> ) 2) Subretinal (8.25E10 vg; 150 µl; 3/8/10)
Ripley (female)	Subretinal (8.25E10 vg, 150 µl; 2/2/10)	1) Subretinal (8.25E10 vg, 150 µl; 2/2/10) 2) Subretinal (8.25E10 vg, 150 µl; 3/8/10)
Mercury (male)	Subretinal (8.25E10 vg, 150 µl; 2/2/10)	1) (8.25E10 vg; total volume of 150 µl: 50 µl subretinal; 100 µl went vitreal; 2/2/10) 2) Subretinal (8.25E10 vg, 150 µl; 3/8/10)
Saturn (female)	Subretinal (1.1E11 vg, 200 µl; 2/2/10)	1) Un-injected (choroidal edema & hyphema) 2) (8.25E10 vg, total volume of 150 µl: 100 µl subretinal; 50 µl went sub-RPE; 3/8/10)
Uranus (male)	Subretinal (1.1E11 vg; 200 µl; 2/2/10)	1) (1.1E11 vg; total volume of 200 µl; 20 µl subretinal; 180 µl went choroidal; 2/2/10) 2) Subretinal (1.1E11 vg; 200 µl; 3/8/10)

Source: Study Report #(b) (4) Canine Ipsilateral-3, submitted in Module 4.2.3.2 of the BLA

#### Key Evaluations and Assessments:

##### In-life:

- Mortality/morbidity: Twice daily
- Clinical observations and appetite: Once daily
- BWs: Prior to first injection and at least once monthly thereafter
- Ophthalmic examinations: Prior to first injection and 1.5 months after re-administration for all animals and prior to sacrifice for 3/5 dogs.
- Specific ophthalmic examinations for inflammation and any flattening of the retina due to detachment: Multiple time points following the first and second injections
- ELISA analysis for anti-AAV2 capsid NAb and anti-AAV2 antibodies (serum and intra-ocular fluids): Baseline (pre-injection) and several time points following first and second injections (up to 10 weeks post-first injection)
- T cell response to the AAV2 capsid and the RPE65 protein (Luminex-based multiplexed cytokine analysis in PBMCs, splenocytes, and lymph node cells and ELISPOT in PBMCs): 10 weeks post-first injection
- Visual behavior (navigation, nystagmus, pupillary light reflexes): Baseline prior to first injection and 6 weeks post-second injection
- Full field ERGs: Pre-injection (baseline) and 5-6 weeks post-second injection

##### Terminal (2.5 months post-first injection):

- Gross pathology
- Ocular histopathology: Eyes, optic nerve (for Ripley, Saturn, and Uranus)
- Immunofluorescence to detect the presence and location of RPE65 protein in the eyes

*Key results:*

- There were no adverse effects on clinical observations, BWs, appetite, and gross pathology parameters.
- Ophthalmic examinations: There were minimal inflammatory changes in the eyes and the retinal detachments resolved by the first post-operative exam (24 hours later).
- Antibody analysis:
  - Serum and intra-ocular fluids: Anti-AAV2 capsid NABs were not detected in the serum or in the anterior chamber of the eye at baseline (titer of 1:1). Titers (1:100-1:1000) were detected in serum in all animals following first injection. NAB titers (1:100-1:1000) were detected in the anterior chamber and vitreous in 4/5 animals (except for Mercury). These titers were generally consistent between the left and right eye, except for Saturn, who had detectable anti-AAV2 antibodies only in the ocular fluid of the left eye.
  - Anti-AAV2 capsid antibody results were consistent with those of the NAB assay. Antibody subclass analysis showed a predominantly Th2-driven response (reflected by higher IgG2 than IgG1 levels).
- T cell immune response: Cytotoxic T cell responses directed against either the AAV2 capsid or the expressed human RPE65 protein did not develop following subretinal injections.
- Visual behavior: All animals showed improved navigation at 10 weeks post-first injection (6 weeks post-second injection). The greatest improvement was observed in Saturn and Mercury. The baseline pupillary responses of all dogs were slow and diminished compared to normal-sighted dogs. However, the post-injection pupillary responses appeared to improve in all animals.
- ERGs: Baseline ERG responses were flat. All eyes had detectable ERG signals post-second injection, except the left eye of Uranus and the right eye of Ripley and Mercury. Responses in the left eyes (which received two separate subretinal injections) were greater than those in the right eyes (which received one injection) in all dogs, except Uranus and Venus. The lowest response was noted in Ripley, who was the oldest dog at the time of first injection (8.5 months old). The highest ERG responses were found in the left eyes of Saturn and Mercury (4 months old).
- Ocular histopathology (three of five animals): For all three animals, there was focal injury at, and surrounding the retinotomy site, but otherwise normal retinal anatomy/layers were present. In one focal region of the left eye of Uranus (who also was inadvertently injected via the choroidal route in addition to subretinal for the first injection), inflammatory cells (mononuclear cells) were present in the choroid.

In all other eyes of all animals there were rare lymphocytes in the vitreous and occasional macrophages in the subretinal space. For all eyes, there were no inflammatory cells in any optic nerve, and all layers of the retina had normal thickness/cell counts.

- RPE65 immunofluorescence: RPE cells (only) were positive for RPE65 protein in all eyes in the region<sup>13</sup> exposed to AAV2-hRPE65v2, except for the left eye of Uranus (due to injection via the choroidal route). The strongest fluorescence was found in the eyes that showed the greatest ERG responses. There were fewer RPE65-positive RPE cells in the eyes that showed reduced ERG responses. There was no RPE65-specific fluorescence detected in the optic nerve from any of the three animals.

**Comment:**

- While the first injection in the left eye of Uranus went to choroidal, this left eye also received a second injection approximately one month later, the Study Director did not explain as to why RPE65 protein was not detected in the left eye of Uranus.

**Summary from the study report:**

- There were no signs of systemic toxicity following repeat subretinal injection of AAV2-hRPE65v2.
- There was no evidence of local toxicity following repeat subretinal administration of AAV2-hRPE65v2 as assessed by ophthalmic examinations and histopathology.
- The visual behavior of all dogs improved following subretinal injection of AAV2-hRPE65v2. The level of improvement for each animal generally correlated with their ERG response, as well as with age at the time of the first injection (i.e., the younger the age, the greater the improvement).
- Development of NAbS to the AAV2 capsid was detected both systemically (serum) and locally (anterior chamber and vitreal fluids). No cytotoxic T cell response directed against either the AAV2 capsid or the expressed human RPE65 protein was detected.

**APPLICANT'S PROPOSED LABEL**

Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14)<sup>14</sup>.

Section 12.3 ('Pharmacokinetics – Nonclinical data') should be revised with the appropriate wording to accurately reflect the available nonclinical data.

<sup>13</sup> Per the ocular pathology report, the region(s) of the original detachment could be identified in the posterior eye cups by a faint mark delineating the edge of bleb formed by the subretinal injection.

<sup>14</sup> Pregnancy and Lactation Rule (PLLR), at:

<http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/actsrulesregulations/ucm445102.htm>.

Section 13 ('Nonclinical Toxicology') should be revised with the appropriate wording to accurately reflect the available nonclinical data.

**CONCLUSION OF NONCLINICAL STUDIES**

Review of the nonclinical studies did not identify any safety concerns that could not be adequately addressed in labeling. The nonclinical data support approval of the license application.

**KEY WORDS/TERMS**

Gene therapy, LUXTURN<sup>TM</sup>, AAV2-hRPE65v2, AAV2, subretinal, RPE65, retinal dystrophy, immunogenicity, (b) (4) dogs, *RPE65*<sup>-/-</sup> mice, *rd12* mice, non-human primates