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MONOGRAPH

rAAV2-CBSB-hRPE65

Ophthalmic Gene Therapy
Treatment of Leber's Congenital Amaurosis

rAAV.hRPE65v2
AAV2.RPE65

Recombinant adeno-associated serotype 2 (rAAV-2) virus vector that carries the *RPE65* gene, encoding a retinal pigment epithelium (RPE)-specific human retinoid isomerohydrolase, under the control of a cytomegalovirus immediate early enhancer and a chicken β-actin promoter

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SUMMARY

Leber's congenital amaurosis (LCA) is a group of genetic disorders that produces defects in the retinal epithelium photoreceptors of the eye. It manifests at an early age, resulting in impaired vision and ultimately blindness. It is estimated that as many as 10% of children attending schools for the visually impaired suffer from this type of retinal disease. Lack of retinoid isomerohydrolase, a protein essential for correct photoreceptor function, due to a genetic mutation has been cited as a major cause of type 2 LCA (LCA2). Preclinical evidence from rodent and canine models has shown that gene therapy using a recombinant adeno-associated virus (rAAV) to deliver functional retinoid isomerohydrolase protein can successfully restore some degree of visual function in animal models of LCA and can prevent associated retinal degeneration. Following this preclinical evidence, the University of Pennsylvania in collaboration with Applied Genetic Technologies has produced an rAAV vector that expresses the human protein under the control of a chicken β-actin promoter. This construct is currently undergoing phase I and II clinical trials in patients with LCA2. Preliminary results indicate that subretinal injection of rAAV2-CBSB-hRPE65 does not produce any discernable adverse immunogenic response and can improve visual function over the long term in young adults diagnosed with LCA2.

BACKGROUND

Leber's congenital amaurosis (LCA) is a group of rare, early-onset, autosomal recessive hereditary disorders characterized by severe retinal dystrophy, nystagmus, no pupillary response and flat signaling on electroretinogram (ERG). LCA that leads to gross visual impairment and subsequent blindness in adulthood can affect children as young as 6 weeks old and is usually present before a child reaches 1 year of age. A genetic basis for LCA and related retinal dystrophies has been established and is known to currently involve 400 mutations across 14 candidate genes involved in diverse developmental and functional visual processes, such as photoreceptor morphogenesis (*CRB1, CRX*), phototransduction (*AIPL1, GUCY2D*), vitamin A cycling (*LRAT, RDH12, RPE65*), guanine synthesis (*IMPDH*1) and outer-segment phagocytosis (*MERTK*) (1). The involvement of a wide number of mutations leads to genetic heterogeneity among patients, presenting problems for therapeutic targeting, although there is a degree of commonality and microarray screening can identify the mutations present (2, 3).

The *RPE65* gene encodes a retinal pigment epithelium (RPE)-specific isomerohydrolase found in the endoplasmic reticulum of RPE cells that is involved in the isomerization of 11-*cis*-retinal from *all-trans*-retinal, a key step in the production of the visual pigment rhodopsin via the retinoid cycle (4-6). Frameshift mutations in the *RPE65* gene result in the production of nonfunctional truncated retinoid isomerohydrolase proteins and subsequent disruption of rhodopsin production and photoreceptor function, and have been highlighted as one of the causes of the early onset of LCA in approximately 6% of cases (4, 7). Specific markers of *RPE65* mutations include poor vision, photophobia, lack of night blindness, long-term stability in longitudinal studies of visual performance with transient improvements, and lack of photoreceptor disc recycling (5).

Gene therapy as a candidate to overcome genetic mutations and restore loss of function has become an attractive prospect for future therapy. Gene therapy is particularly appropriate for use in visual defects because the eye harbors natural advantages over other somatic targets for transgene delivery. Foremost, it is easily accessible and the subretinal space provides a location where the therapy can be administered without affecting the central nervous system. Additionally, the unique structure of the eye and its blood–retinal barrier minimizes immunological responses to viral antigens. Furthermore, the administration and progress of the treatment can be easily monitored with a multitude of noninvasive examination techniques (8). Adeno-associated virus (AAV) can transduce RPE and photoreceptor cells with very high efficiency, while eliciting a minimal immune response, resulting in long-term expression of the transgene (9).

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rAAV2-CBSB-hRPE65 (AAV2.RPE65, rAAV.hRPE65v2) is a recombinant AAV serotype 2 (rAAV-2) vector that carries the gene for human retinoid isomerohydrolase under the control of the cytomegalovirus immediate early enhancer and the chicken β-actin promoter. It was developed through a collaboration among the University of Pennsylvania, Yale University, the University of Florida and Cornell University. Following encouraging preclinical evidence, the University of Pennsylvania and Applied Genetic Technologies are currently conducting clinical trials of rAAV2-CBSB-hRPE65 in patients with LCA due to *RPE65* mutations (LCA2).

PRECLINICAL PHARMACOLOGY

Initial preclinical studies examining gene therapy as a mechanism to restore retinoid isomerohydrolase function were carried out in utero in an *Rpe65*$^{-/-}$ knockout mouse model of LCA. Knockout of the developmentally regulated *Rpe65* gene, while producing no major effects on retinal structure, resulted in the inability to produce 11-*cis*-retinal and rhodopsin, which can lead to severe visual impairment. When assessed at 1-2 months postinjection, subretinal injection of a recombinant AAV2/1-CMV-hRPE65 vector in utero at E14 had no effect on the development of mice. Immunohistochemistry revealed de novo production of retinoid isomerohydrolase protein in RPE cells that persisted from birth up to 6 months (termination of study). Two ERG parameters that measure visual function, ERG photoreceptor threshold and photoresponse sensitivity, were employed to assess the efficacy of the therapy. Vector injection increased photoreceptor activation threshold amplitudes in treated *Rpe65*$^{-/-}$ mice to levels similar to those seen in wild-type (*Rpe65*$^{+/+}$) mice (−2.9 and −3.9 ± 0.3 log scot cd.s/m^2, respectively; n = 8). No improvement was seen in untreated control *Rpe65*$^{-/-}$ mice or the contralateral noninjected/sham-injected eye (−0.6 ± 0.3 and −0.5 log scot cd.s/m^2, respectively; n = 8). AAV2.RPE65 injection also increased photosensitivity in the mutant mice, producing a fast leading-edge peak of 4 ms comparable to wild-type mice (3.39 and 3.2 ± 0.2 log scot/cd/m^2/s^3, respectively; n = 6). Conversely, in the contralateral control eye or untreated *Rpe65*$^{-/-}$ mice, photosensitivity was not improved, displaying a slow leading-edge peak of 10 ms (1.68 and 2.0 ± 0.3 log scot/cd/m^2/s^3, respectively; n = 6) (10). When compared to untreated mutants, young adult injected *Rpe65*$^{-/-}$ mice (P30 and P75) also displayed highly significant improvements in photoreceptor activation threshold (−1.9 ± 1.2 vs. −0.2 ± 0.3 log scot cd.s/m^2) and photoresponse sensitivity (2.8 ± 0.6 vs. 1.7 ± 0.3 log scot/cd/m^2/s^3). Improvements in rod-specific (scotopic) ERG parameters were observed (0, 189 and 378 μV, respectively, for sham-treated/untreated *Rpe65*$^{-/-}$, virus-treated *Rpe65*$^{-/-}$ and wild-type C57BL/6 mice) in 4 of 10 young *Rpe65*$^{-/-}$ mice injected with an optimized form of AAV2.RPE65. Additional improvements were noted in the b wave amplitudes (secondary retinal neurons) of *Rpe65*$^{-/-}$ mice treated with AAV2.RPE65 (0.5, 59 and 233 μV, respectively, for sham/untreated *Rpe65*$^{-/-}$, virus-treated *Rpe65*$^{-/-}$ and wild-type C57BL/6 mice) (11).

Biochemical analysis of retinal rhodopsin concentrations in AAV2.RPE65-injected eyes from both fetal and postnatal *Rpe65*$^{-/-}$ mice revealed a significant increase in rhodopsin concentration (67 and 57 pmol, respectively). Peak expression in one fetally treated mutant reached 190 pmol, which is approximately 66% of that seen in C57BL/6 wild-type mice (10). In a naturally occurring *Rpe65*-null mouse, rd12, improvements were evident in visual acuity concomitant with de novo expression of *Rpe65* in the photoreceptor layer in eyes that received subretinally injected AAV2.RPE65 (11).

Although these studies demonstrate improvement in visual sensitivity, others noted that in the *Rpe65*$^{-/-}$ murine model, despite long-term expression of the transgene, these improvements may be transient (12). Similar studies in neonatal rd12 mice using a recombinant AAV serotype 5 vector (AAV5-CBA-hRPE65) demonstrated a 66% recovery in visual function and prevention of the onset of retinal deterioration normally observed at 3 weeks of age, suggesting that early therapy could prevent disease progression associated with *RPE65* mutations later on in life (13).

In 2001, the first large-mammal investigation using rAAV to restore loss of visual function was performed in a naturally occurring canine model (briard/briard beagle) that displays severe and early-onset visual impairment and blindness that mimics human LCA2. These dogs harbor a homozygous 4-bp deletion in the coding sequence of the *RPE65* gene, resulting in the production of a nonfunctional truncated protein. Cultured RPE cells from *RPE65*$^{-/-}$ dogs were injected with rAAV virus serotype 2/2 carrying chicken β-actin promoter/CMV enhancer-driven wild-type canine *RPE65* cDNA. At 10 days after treatment, immunocytochemistry and Western blotting showed a marked increase in retinoid isomerohydrolase protein (restricted to the RPE) comparable to levels observed in untreated *RPE65*$^{+/+}$ cells, while in untreated *RPE65*$^{-/-}$ cells protein was notably absent. In *RPE65*$^{+/+}$ dogs the amplitude of photoreceptor cell traces increased following increasing energy stimulation. In contrast, the threshold stimulus for a trace response in *RPE65*$^{-/-}$ dogs required a 4.5-log increase in energy stimulation strength. Moreover, energy stimulation that would elicit a saturation response in wild-type animals failed to produce any recordable ERG activity in 4-month-old *RPE65*$^{-/-}$ dogs (n = 3). Following subretinal injection of AAV2.RPE65 in one *RPE65*$^{-/-}$ dog, photoreceptor response was markedly improved by 4-log units to levels similar to that in *RPE65*$^{+/+}$ dogs. In three subretinally injected *RPE65*$^{-/-}$ eyes photoreceptor trace amplitudes were increased to 27, 38 and 58 μV compared to pretreatment (14 ± 3.4 μV), showing a marked improvement over untreated eyes to response levels approximately 16% of that in wild-type dogs (249 ± 95 μV). Pupillary constriction in response to stimulus, a measure of the transmission of retinal processing to higher visual centers, was greatest in wild-type dogs and lowest in untreated *RPE65*$^{-/-}$ dogs, with virally treated *RPE65*$^{-/-}$ dogs displaying a response somewhere between the two (14).

Behavioral testing of the animals involved in the above study was performed 4 months postinjection to assess visual function. Treated *RPE65*$^{-/-}$ dogs were rated as displaying normal vision under daylight conditions and displayed object avoidance behavior under dim light conditions, but only on the side of the subretinally injected eye. The same animal that received an intravitreal injection in the opposite eye was unable to avoid objects on the ipsilateral side. Comparably, the untreated *RPE65*$^{-/-}$ dog did not show any improvement in avoidance behavior. Overall, this study was the first to demonstrate restored function in a large-mammal model using retinal gene therapy and prompted the possibility that a similar method could be used to treat human LCA2 caused by *RPE65* mutations (14).

This study was later extended to include additional animals and long-term investigation over a 3-year period. In addition, nystagmus and disease-specific accumulation of lipid droplets in the RPE were ablated at 10 weeks following subretinal injection of AAV2.RPE65 in *RPE65*$^{-/-}$ dogs (n = 11). Furthermore, 10 of 11 dogs displayed signs of improved vision, ERG amplitude responses improved in the treated eye, with 6 of 11 dogs showing photoreceptor threshold responses comparable to wild-type controls and photoreceptor amplitudes approximately 28% of normal (dark-adapted) and 32% of normal (light-adapted). Upon reexamination of treated animals 9 months postinjection, improvements in visual function were stable (15). Following subretinal administration of AAV2.RPE65, photoreceptor ERG traces showed a range of amplitudes in 23 of 26 eyes from *RPE65*$^{-/-}$ dogs compared to untreated controls. Another study further confirmed marked and mid- to long-term improvements in irregular eye movements and photoreceptor function (26-60% of wild-type controls) in three 2-month-old *RPE65*$^{-/-}$ dogs following subretinal injections (12). In addition, two dogs from the initial study were assessed yearly for 3 years to ascertain the long-term effects of subretinal injection. In both animals improvements in visual function, ERG response and photoreceptor sensitivity were maintained and stable. Overall improvements in vision following a single subretinal injection of AAV2.RPE65 have been shown to persist over the long term for more than 7 years (12, 14, 16). The RPE of untreated, control-treated or intravitreally injected *RPE65*$^{-/-}$ dogs had no measurable 11-*cis*-retinal, whereas low levels of 11-*cis*-retinal were detectable following subretinal injection of AAV2.RPE65 in *RPE65*$^{-/-}$ dogs, although this was limited to the site of injection (16).

SAFETY

Possible off-target effects have been raised as a major concern of gene therapy, not only due to genome manipulation, but also due to the nature of gene delivery. Viruses, with their ability to infect a wide range of human cell types, have a natural advantage when it comes to transgene delivery to the cell nucleus. However, major obstacles to viral therapy include random genome integration that may disrupt the existing genome or elicit transgene silencing, uncontrolled replication, which could ultimately lead to complications owing to vast overexpression in unwanted tissues, and adverse events (AEs) via immune responses to viral capsid proteins. Recombineered AVV vectors lack the viral replication and capsid protein-coating genes that prevent uncontrolled replication once introduced into a system, so that once inside the host cell the virus is unable to enter the lytic cycle or trigger an immune response. Moreover, rAAVs are incapable of genome integration, thereby minimizing concerns over random integration effects or transgene silencing. As mentioned earlier, the use of these vectors is particularly promising in the visual system due to the architecture of the eye. The effective isolation of the eye also permits smaller doses of vector genome to be used compared to when used systemically in gene therapy (17).

The safety and biodistribution of AAV2.CBA.RPE65 were first assessed in canine and murine models. No systemic adverse effects were recorded in either model, and mild, reversible ocular inflammation up to 3 months after injection was seen in dogs as a consequence of surgery. Moreover, examination of blood, optic nerve (n = 21 of 22) and brain visual centers (n = 15 of 15) revealed a minimal presence of vector DNA outside the eye, although in two isolated cases vector sequences were present in the heart and diaphragm of one dog and the diaphragm of a second dog. Retinal trauma was observed at the site of injection, accompanied by retinal thinning when high doses were administered. Dose–response curves in the canine model indicated that the highest 1.5-log unit range of vector doses was effective (18, 19). Several earlier studies in dogs did note some immunogenic AEs related to viral administration, although they were due to contaminated viral preparations, suggesting that extremely stringent protocols must be followed during viral preparation and purification (14).

Following the successful restoration of visual function and lack of AEs in rodent and canine LCA models, safety experiments were performed in cynomolgus monkeys (N = 17), a nonhuman primate model with human-like foveate retinal structure. One week following a single subretinal injection of vector genome rAAV2-CBSB-hRPE65, inflammation due to surgery had ablated. Vector sequences present in the eye and optic nerve at 1 week postinjection were not detected at 3 months; two monkeys, however, did show detectable levels of vector DNA in skeletal thigh muscle and lung. No significant changes in dark-adapted ERG parameters were recorded between injected and noninjected control eyes (n = 11). No retinal thinning was observed in response to a dose–response curve, although in some animals abnormal foveate architecture was observed. This study concluded that the use of rAAV2-CBSB-hRPE65 in a nonhuman primate model produced little or no AEs at 4.5×10^{12} vector genomes (vg) and could provide a promising vehicle for future gene therapy in a human model of LCA (20).

Safety assessment in human clinical trials is under way, and to date, no long-term AEs, immunogenic reactions or toxicities have been noted for up to 3 years following subretinal injection. One study did suggest that foveal injections should be avoided to prevent damage to foveate photoreceptors (17). Of note is that there has been no standardized approach in all preclinical and clinical trials. Differences in the rAAV construction, injection volumes, sites of retinal injection and dose have been reported. In addition, a range of baseline values in sensitivity, acuity and degree of retinal deterioration have been noted among patients, with some responding better than others to treatment. The vast differences between studies can make interpretation of efficacy confounding, although it is clear that at least up to 3 years following treatment no major safety issues have been raised.

CLINICAL STUDIES

Following the successful application of AAV2.RPE65 gene therapy in preclinical studies, particularly in the large-mammal model, the University of Pennsylvania and collaborators at the University of Naples and Applied Genetic Technologies are currently conducting phase I clinical trials to assess the safety and efficacy of rAAV2-CBSB-hRPE65 in treating patients with LCA.

The only currently active nonrecruiting study is a nonrandomized, open-label, dose-comparison study being carried out by the Children's Hospital of Philadelphia (21). The study commenced in September 2007 and will run for 5 years. The primary outcome measures are safety and tolerability, while secondary outcome measures will examine changes in visual function. Twelve patients were recruited, with eligibility criteria including age

> 8 years, visual acuity ≤ 20/160 or visual field less than 20° in the eye to be injected, and confirmation of LCA due to *RPE65* mutations. Patients received an injection of 1.5 × 10^{10} vg (low dose) of rAAV.hRPE65v2 in a volume of 150 µL of phosphate-buffered saline supplemented with Pluronic F-68 NF Prill Poloxamer 188 into the subretinal space, which created a localized dome-shaped retinal detachment. Initially, 3 patients 19-26 years of age received an injection in the eye with the most compromised visual function, and outcome was assessed over a 1-year period. There were no serious AEs as a result of virus delivery and any initial changes in retinal appearance as visualized by OCT or ophthalmoscopy were resolved or had no discernable effect on visual function. There was no evidence of vector presence in other tissues or of immune responses. Measurements of pupillometry in response to a dim 0.04 scotopic lux stimulus (200 ms) showed that all patients had negligible pupil response (PLR) prior to treatment, compared to controls (2 mm constriction). Increasing the magnitude of the stimulus by a factor of 250 did not improve PLR in untreated patients. Following viral delivery, the PLR of the injected eye of each patient displayed a highly significant 3-fold improvement in sensitivity over the noninjected eye, although the time course and stimulus intensity required for a notable change varied among patients (4.75 months and 10 lux, 2.75 months and 10 lux, and 1 month and 0.04 lux, respectively, for patients 1, 2 and 3) (22). Follow-up examinations 1.5 years following treatment suggested that the majority of positive PLR changes in amplitude and velocity occur within the first 4 months following injection, with very gradual increases over time, and that these positive changes persist over the long term (23). Two weeks following low-dose rAAV.hRPE65v2 injection, patients showed notable improvements in visual acuity. In all patients average visual acuity logMAR (logarithm of the minimum angle of resolution) scores for hand–motion recognition significantly improved by 0.28, 0.34 and 0.45 ($P \leq 0.001$, $P = 0.002$ and $P \leq 0.001$, respectively), equivalent to improvements from 0 letters to 3, 3.5 and 4.5 lines, respectively, on a Snellen eye chart. Visual field area improvements were also recorded for each patient, with a 2-4-fold increase in degree for the treated eye. An additional sign of treatment success included improvement in nystagmus, with decreases in both monocular and binocular amplitude and frequency. One patient who was previously unable to negotiate a 14-object obstacle course could successfully follow arrows on the course following treatment and all improvements persisted when examined 6 months following injection (22). Subsequent follow-up of these 3 patients 13.5 months following injection showed sustained improvement in visual function and confirmed a lack of AEs (24, 25). A further follow-up at 1.5 years following injection supported these findings. In addition, fundus photography and OCT showed an unchanged macular profile and thickness in two of the patients compared to baseline (23). Following treatment with low-dose rAAV.hRPE65v2, all patients demonstrated improved ocular motility, showing constant and symmetric eye motion, decreases in monocular and binocular nystagmus frequency in the primary position (average baseline frequency = 1.5, average frequency at 60 days = 1.17, average frequency at 1.5 years = 0.84 [injected eye measurements]) and a significant reduction in binocular nystagmus amplitude in primary gaze (average amplitude at baseline = 7.6°, average amplitude at 60 days = 4.1°, average amplitude at 1.5 years = 1.9° [injected eye measurements]). In addition, the initial improvements in subjective measures of visual acuity recorded following injection (22) showed further marked improvements and were ongoing at 1.5 years postinjection, as measured by early treatment diabetic retinopathy study (ETDRS) (20/640, 20/459 and 20/167, respectively, for patients 1, 2 and 3) and positive changes in logMAR values (0.21, 0.19 and 0.24 at 1.5 years compared to days 150, 80 and 45, respectively, for patients 1, 2 and 3 [injected eye measurements]). There were no further improvements in visual field parameters, as measured by Goldmann visual field tests, at 1.5 years as compared to the significant improvements observed in the early postinjection stages. The ability to successfully navigate an obstacle course continued to improve at 1.5 years compared to the early-stage assessments for one patient but remained stable for the other two patients, yet still significantly improved compared to the baseline inability to navigate the course (23).

Dose-escalation studies performed over 15.5 months included 9 patients (8-26 years of age) who received a single subretinal injection of either 1.5 x 10^{10} or 4.8 x 10^{10} vg rAAV.hRPE65v2. No AEs were observed and each patient demonstrated signs of subjective and objective improvement in visual function (26). The effect of age on the success of treatment was assessed in 12 patients (8-44 years of age). Dose regimens included low-, medium- and high-dose protocols (1.5 x 10^{10}, 4.8 x 10^{10} and 1.5 x 10^{11} vg, respectively) and patients were assessed over 2 years. At 2 weeks postinjection, all patients reported improved vision in dimly lit environments. Age-independent stable improvements in visual acuity were recorded in the low-dose cohort (n = 3), mid-dose cohort (n = 3) and high-dose cohort (n = 1); other patients showed no improvement (n = 4) and one displayed a decrease in visual acuity. Visual field was improved in the injected eye in all 12 patients and could be closely correlated with, but not limited to, additional postoperative parameters, such as the degree of structurally intact but nonfunctional retina, or the presence of scotomas, which were subsequently obliterated by therapy. Improvements in visual field were also found to be age-dependent, with patients younger than 19 years of age displaying greater gain of function than those over 19 years of age, although this was not surprising based on the progressive retinal degeneration that is characteristic of LCA (27). Interocular comparisons at various time points following injection showed that full field sensitivity to white light was significantly improved in five of seven patients (from the mid- to high-dose cohort), with the youngest patients showing the best response. When pupillary response on exposure to a dim stimulus (0.04 lux, 200 ms) was assessed in 11 patients (mid- to high-dose cohort) all rAAV.hRPE65v2-injected eyes displayed significant PLR amplitude and velocity improvements of > 2 log. One 8-year-old patient showed improvement to levels comparable to normal-sighted individuals. Photosensitivity changes in injected eyes could be correlated with age: 2.2-log units for 8-11 years of age and 1.2-log units for 19-44 years of age ($P = 0.04$). ERG recordings were only possible in three patients, all of whom showed responses to stimulus that were absent prior to injection. Prior to injection, 11 patients tested found completion of an obstacle course extremely difficult, especially in conditions of low light. Following injection, four patients, all young children, demonstrated significant improvement in navigation and time taken to completion when presented with the same obstacle course (26).

A similar rAAV2/2 vector was employed in a clinical study carried out at University College London (28). AAV2/2.hRPE65p.hRPE65 encompasses the human RPE65 coding sequence driven by a 1400-bp fragment of its own promoter rather than the chicken β-actin promoter (29). This vector should effectively target RPE cells that would normally express the endogenous gene but the strength of gene expression will differ from the vector that employs the chicken β-actin promoter. The study, which began in January 2007 and is scheduled to end in 2013, is a single-center, open-label, dose-escalating phase I/II study to assess safety and efficacy. Primary outcome measures include intraocular inflammation, and secondary outcome measures include visual function. All 3 patients (17-23 years of age) recruited had early-onset LCA with confirmed *RPE65* mutations. Prior to treatment, all three patients had no or severely limited vision in conditions of dim light but retained some degree of retinal function despite advanced retinal degeneration. No AEs or immune responses associated with RPE65 protein or AAV were recorded following subretinal injection of the virus. Over an initial 6-month period, none of the patients showed any significant improvement in visual acuity compared to baseline, although retinal detachment following surgery can take up to 6 months to resolve. At 12 months, 1 patient showed a slight but not significant improvement in acuity, 1 did not show any improvement and 1 has so far not reached the 12-month stage. No flash or pattern ERG activity was displayed following treatment and patient 2 displayed signs of high-amplitude nystagmus which was not resolved following viral administration. Retinal function, as measured by microperimetry, was improved in one patient, who showed an increase in retinal sensitivity to bright light by a factor of 25. The same patient also showed increased sensitivity by a factor of 100 in dark-adapted perimetry. At baseline under dim light this patient took 77 s to complete an obstacle course and concurred 8 errors, whereas following treatment the patient completed the course with no errors in a significantly shorter time of 14 s. Neither of the other two patients showed any improvement compared to baseline levels.

An open-label phase I study is being conducted at the University of Florida and the University of Pennsylvania (30). The study began in 2007 and will run for 15 years. Primary outcome measures are to assess toxicity and ocular safety, and the secondary outcome measure is visual function. The study contains 3 cohorts of 3 patients with LCA due to *RPE65* mutations, the first 2 cohorts consisting of patients aged > 18 years. One cohort (21-24 years) received a subretinal injection of rAAV2-CBSB-hRPE65 (5.96×10^{10} vg; mid-dose) and were assessed by follow-up examinations at 30-90 days. There were no AEs, although OCT revealed retinal thinning in patient 1. There was no significant improvement in foveal visual acuity measured on the Snellen scale at 90 days postinjection (20/317, 20/138 and 20/191, respectively, in patients 1, 2 and 3) compared to baseline (20/240, 20/195 and 20/283, respectively). Dark-adapted visual full-field sensitivity tests revealed that prior to treatment patients displayed decreased sensitivity to light 3-4 orders of magnitude lower than for normal-sighted individuals. After injection all patients displayed significant increases in light sensitivity threshold compared to baseline measurements (17). Increased sensitivity in the treated retinal region in patient 1 was determined to be mediated by increased rod function, while that for the other two patients was mediated by both rods and cones (31).

Follow-up of this study at 1 year postinjection further confirmed that no AEs or abnormal immune responses were evident in the three patients. At 90 days, the antigen-specific (AAV2 capsid) lymphocyte proliferation response (ASR) of 1 patient was slightly higher than the threshold values for no immune response (17); however, by 1 year postinjection the ASR was unchanged from baseline values. At 1 year after surgery the magnitude, retinal extent and photoreceptor type involvement of the increase in visual sensitivity measured at 90 days did not show any further improvement (32).

Further experimentation was performed on one of the patients in this study following an unusual gain of visual function that could not be correlated to any of the previously measured parameters. This patient appeared to show greater sensitivity to light without any measurable increase in visual acuity or light sensitivity. Investigation into foveal fixation over a range of dim targets revealed that the foveal cone sensitivities in the control and treated eyes were similar, but that there was a fixational shift to the superotemporal region in the treated eye, creating a pseudofovea in an area that was "cone blind" in the untreated eye. The study concludes that this may be a heretofore unseen and unexpected benefit of the treatment (33).

A number of early-phase trials are in the recruitment stage or are currently being planned. Applied Genetic Technologies in collaboration with the Oregon Health and Science University and the University of Massachusetts is conducting a nonrandomized, open-label phase I/II study of the safety and efficacy of rAAV2-CBSB-hRPE65 in patients with type 2 LCA. Recruitment began in December 2008 and the study will run for 16 years, with primary outcome measures of ocular safety expected by December 2011. Patients will be grouped by age (8-17 and > 17 years of age) and will receive either a low or high vector dose (34). Two further studies examining the safety and efficacy of AAV.hRPE65 in treating LCA are planned but are not yet recruiting. The first is a 7-year, open-label, dose-comparison phase I/II study due for completion in January 2017 and will be conducted in Israel at the Hadassah Medical Organization. The study will initially examine safety in 2 cohorts (3 patients each, > 18 years of age), each receiving a different concentration of vg. Once safety has been established, a third cohort of 4 patients aged 8-18 years will be included (35). The second trial, a 2-year, open-label phase III safety/efficacy study to assess the use of rAAV.hRPE65v2 to treat type 2 LCA in children (> 3 years of age) and adults is being conducted by the Children's Hospital of Philadelphia in collaboration with the University of Iowa. The primary outcome measures will be PLR light sensitivity, and secondary outcome measures will assess additional changes in visual function, structural changes and AEs (36).

SOURCE

Applied Genetic Technologies (US).

DISCLOSURES

The author states no conflicts of interest.

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