Choroideremia Gene Therapy Phase 2 Clinical Trial: 24-Month Results

BYRON L. LAM, JANET L. DAVIS, NINEL Z. GREGORI, ROBERT E. MACLAREN, ANIZ GIRACH, JENNIFER D. VERRIOTTO, BELEN RODRIGUEZ, POTYRA R. ROSA, XIAOJUN ZHANG, AND WILLIAM J. FEUER

• PURPOSE: To report the final results of a phase 2 high-dose gene therapy clinical trial in choroideremia.

• METHODS: Design: Phase 2 clinical trial. Participants: Six men (aged 32-72 years) with genetically-confirmed advanced choroideremia. Patients received subfoveal injection of AAV2-REP1 (10^{11} genome particles in 0.1 mL) in the worse-sighted eye. Outcome Measures: Primary measure was best-corrected visual acuity (BCVA) change from baseline in the treated eye compared to the untreated eye. Secondary endpoints included change from baseline in microperimetry, fundus autofluorescence, and spectral-domain optical coherence tomography (OCT). Safety evaluations included adverse events, viral shedding in body fluids, and vector antibody responses.

• RESULTS: Baseline mean ETDRS BCVA was 65.3 ± 8.8 (SD, range 56-77, 20/32-20/80) letters in the treated eyes and 77.0 ± 4.2 (69-81, 20/25-20/40) letters in the untreated eyes. At 2 years, 1 treated eye improved by 10 letters and another by 5 letters, while 1 untreated eye improved by 4 letters. All other eyes were within 2 letters of baseline. Baseline microperimetry sensitivities in the treated eyes were poor (1.2 ± 2.1 (0, 5.1) dB) and showed no significant change. No serious adverse event occurred. Two patients developed an atrophic retinal hole in a nonfunctioning macular area where baseline OCT showed preexisting thinning. Intraoperative microscope-integrated OCT allowed proper subretinal injection with avoidance of excessive foveal stretching and macular hole formation.

• CONCLUSIONS: Sustained improvement or maintenance of BCVA is achievable in choroideremia with high-dose AAV2-REP1, indicating BCVA is a viable primary outcome in advanced choroideremia. Choroideremia gene therapy delivered with intraoperative OCT has a good safety profile. (Am J Ophthalmol 2019;197:65–73. © 2018 Elsevier Inc. All rights reserved.)

CHOROIDEREMIA IS A RARE X-LINKED RECESSIVE disorder in which gradual vision loss results from mutation or deletion of the *CHM* gene and absence of the *CHM* gene product, Rab escort protein 1 (REP1), essential for intracellular trafficking.[1,2] This protein is expressed in all cells; however, choroideremia clinically manifests only as progressive retinal degeneration, which includes retinal pigment epithelium cell death, photoreceptor degeneration, choroidal atrophy, microcystic retinal edema, and retinal remodeling with outer retinal tubulations.[2–4] Vision loss progresses from nyctalopia in children to visual field constriction in early adulthood and ultimately to near-complete blindness by age 40-50 years.[2,5] There are no current treatments for choroideremia.

Gene therapy is an attractive option in choroideremia because a single mutation is responsible for the disorder, and relative to other organs, the eye is small, easy to access, and relatively less immunologically reactive, a feature known as immune privilege.[6,7] The 1.9 kB *CHM* cDNA is small enough to package within an adeno-associated virus 2 (AAV2) capsid, which has been used successfully as a gene vector in the treatment of inherited retinal dystrophy.[8–10] In animal and in vitro models of choroideremia, *CHM* gene delivery via an AAV vector restores REP1 expression.[11–13] In 2014, MacLaren and associates reported phase I safety and efficacy data for the first-in-human clinical trial in which low-dose AAV2-REP1 10^{10} gp in 0.1 mL was administered subfoveally to 1 eye of 6 patients with choroideremia with the untreated eye as a control.[1] Target protein expression in the retina was enhanced by the addition of a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to the AAV2-REP1 construct (NSR-REP1; Nightstar Therapeutics, London, UK).[1,14] At baseline, choroideremia was advanced, with low best-corrected visual acuity (BCVA) in 2 of 6 patients.[1] Both patients gained, respectively, 21 and 11 letters in the treated eyes at 6 months posttreatment,[1] with improvements sustained at 3.5 years of follow-up.[15] Baseline BCVA was good in 4 of 6 patients and was maintained at 6 months in all 4 patients[1] and at 3.5 years posttreatment in 3 of 4 patients.[15] The decline in BCVA from 6 months to 3.5 years in the fourth patient,

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From the Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida, USA (B.L.L., J.L.D., N.Z.G., J.D.V., B.R., P.R.R., X.Z., W.J.F.); Nuffield Laboratory of Ophthalmology University of Oxford, and Oxford NIHR Biomedical Research Centre, Oxford, United Kingdom (R.E.M.); and Nightstar Therapeutics, London, United Kingdom (A.G.).

Inquiries to Byron L. Lam, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, 900 NW 17 St, Miami, FL 33136 USA; e-mail: blam@med.miami.edu

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who had a surgical complication leading to a lower dose of vector, was likely owing to degeneration in the fovea.[15]

Recently, Dimopoulos and associates reported results of a phase 1 trial conducted in Alberta, Canada, using the same vector at a higher dose of subfoveal 10^{11} gp AAV2-REP1 in 6 patients with choroideremia.[16] One subject had a BCVA gain of 15 letters in the treated eye from baseline measured at 24 months, while 1 subject had a loss of 8 letters in the treated eye related to a serious adverse event of a localized intraretinal immune response, resulting in loss of spectral-domain optical coherence tomography (SD-OCT) outer retinal structures. Subsequent review of the surgical video revealed that during the initial retinal penetration by the 41 gauge cannula and subsequent injection of viral vector, a small amount of subretinal, intraretinal, and vitreous hemorrhage was released, and several air bubbles up to 1000 μm in diameter were injected into the subretinal space during vector injection. Interestingly, 1 of the 6 untreated eyes had an improvement of greater than 15 ETDRS letters, and the authors stated the suggesting improvement in visual acuity should likely not be used as a primary outcome for future choroideremia gene therapy trials.

The current 24-month phase 2 trial (NCT02553135) was conducted in Miami, Florida, USA, and uses the same high-dose AAV2-REP1 (10^{11} gp, 0.1 mL) as the trial conducted in Alberta, Canada.[16] In contrast to the Alberta study[16] and the MacLaren study,[1] improved safety enhancements to facilitate subretinal gene delivery using an automated foot pedal–controlled system for injecting the vector slowly under real-time visualization with microscope-integrated intraoperative OCT (MIOCT) guidance was used in the current study to allow AAV-REP1 injection into the correct tissue plane and to avoid excessive stretching of the foveal tissue.[17] This MIOCT methodology advancement in the current study resulted in no serious surgical complications such as those encountered in the 2 earlier studies. In addition, similar to the MacLaren study[1] but in contrast to the Alberta study,[16] we found no significant BCVA improvement over time in any of the untreated eyes, indicating that improvement in BCVA could be used as a viable primary outcome for future choroideremia gene therapy trials for patients with advanced choroideremia. Further, we report safety data on the immunogenic responses of the AAV-REP1 vector in serum as well as viral shedding in blood, tears, saliva, and urine, which are not reported in the MacLaren study[1] and the Alberta study.[16]

METHODS

• **STUDY DESIGN AND INTERVENTION:** This 24-month, phase 2, open-label, high-dose gene therapy clinical trial for choroideremia (NCT02553135) was designed to assess the efficacy and safety in patients with advanced choroideremia and was conducted at the Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida, USA. The protocol adhered to the tenets of the Declaration of Helsinki, was compliant with the Health Information Portability and Accountability Act (HIPAA), and was approved by the University of Miami Human Subjects Research Committee. All patients signed an informed consent form before being screened for the study. All patients had genetically confirmed choroideremia by a CLIA certified laboratory. For each patient, the eye with worse BCVA was selected as the treatment eye. If vision loss was symmetrical between the 2 eyes, patients were permitted to choose the eye to receive treatment. The fellow eye was used as the study control. To reduce the risk of immune reaction to the AAV2-REP1 (10^{11} gp, 0.1 mL) vector, patients received oral corticosteroid for a total of 21 days: prednisone 1.0 mg/kg/day up to maximal dose of 80 mg daily starting 2 days prior to vector injection for 10 days, followed by 0.5 mg/kg/day for 7 days, then 0.25 mg/kg/day for 2 days and 0.125 mg/kg/day for 2 days.

• **SURGICAL PROCEDURE:** On day 0, standard 23 gauge 3-port pars plana vitrectomy was performed under general anesthesia with an OPMI Lumera 700 microscope (Carl Zeiss Meditec, Inc, Dublin, California, USA). Elevation of the posterior hyaloid was confirmed by staining with triamcinolone acetonide (Triesence; Alcon, Fort Worth, Texas, USA). The retina was separated from the retinal pigment epithelium in the macula by injection of a small amount of balanced salt solution (BSS; Alcon, Fort Worth, Texas, USA) using a DORC (Dutch Ophthalmic Research Center, Netherlands) 23 gauge/41 gauge retractable subretinal injector and the viscous fluid injection (VFI) function of a Constellation vitrectomy console (Alcon, Fort Worth, Texas, USA). The AAV2-REP1 vector (Nightstar Therapeutics Ltd, London, UK) consisted of a chicken β-actin promoter, the *CHM* cDNA, and a WPRE from the same batch as used in the previous study.[1] The vector had been subjected to annual potency testing of REP1 expression and prenylation activity against Rab substrates using a standardized protocol.[18] The surgical technique, however, incorporated an automated injection system for the vector that had not been used in the previous study. A dual-bore 23 gauge DORC subretinal cannula ending in a 41 gauge Teflon tip was mounted on the loaded syringe and used for injection of BSS into the subretinal space. The vector solution (300 μL) was loaded into a plungerless 1 mL BD Luer-Lok polycarbonate syringe (Becton, Dickinson and Company, Franklin, New Jersey, USA) and attached to a proprietary adaptor and tubing set connected to the Constellation VFI port.[19] Preserved areas of retina were targeted with up to 0.10 mL of AAV2-REP1 at a concentration of 10^{11} genome particles per 0.1 mL suspension. In 5 of 6 patients (Patients 502-506) the Zeiss Lumera

Rescan MIOCT function permitted confirmation of subretinal injection with expansion of the subretinal bleb and monitoring of the foveal region for thinning or hole formation. This revised MIOCT-assisted surgical technique is described in detail elsewhere.[17]

• **STUDY ENDPOINTS:** The patients were followed for the planned duration of 24 months: days 1 and 7, and months 1, 3, 6, 9, 12, 18, and 24. The primary endpoint was the change from baseline in BCVA in the treated eye compared to the untreated eye. Secondary endpoints included the change from baseline in the treated eye compared to untreated eye in central visual field by microperimetry, contrast sensitivity, color vision, fundus autofluorescence (FAF), and SD-OCT assessments. Safety evaluations included testing for viral shedding by polymerase chain reaction (PCR) amplification of vector genomes in blood, tears, saliva, and urine at screening and at day 1, day 7, month 1, month 3, and month 6. Immunogenicity of the AAV-2 vector was assessed by ELISA assay of anti-AAV-2 neutralizing antibody responses in serum at screening and at day 1, day 7, month 1, month 3, and month 6 and by ELISPOT assay of T cell–mediated immune responses at screening and at day 1, day 7, month 1, month 3, month 6, and month 12. Vital signs, blood chemistry, and C-reactive protein were taken at screening and at various time points during the study.

• **PATIENTS:** Inclusion criteria required patients to be adult men with an ocular phenotype of choroideremia, a confirmed disease-causing variant in the gene encoding REP1 protein, and clinically visible progressive disease within the macular region. Patients were also required to have a baseline BCVA in the treatment eye equal to or worse than 78 ETDRS (Early Treatment Diabetic Retinopathy Study) letters (Snellen equivalent 20/32, decimal 0.63, logMAR 0.2) but better than or equal to 34 ETDRS letters (Snellen equivalent 20/200, decimal 0.10, logMAR 1.0). Exclusion criteria included a history of amblyopia in the treatment eye, any genetic retinal disorder other than choroideremia, grossly asymmetric disease or any ocular morbidity that confounded use of the fellow eye as a long-term comparator, unwillingness to use barrier contraception, or contraindication to use of medications or contrast agents such as oral or topical corticosteroids, proton pump inhibitor, fluorescein, indocyanine, anesthetic agents, and mydriatic agents. Patients also were excluded if any other significant ocular or nonocular disease or disorder would put the patient at risk while participating in the study (such as a history of gastric ulcer) or might influence the results of the study or the patient's ability to participate in the study. Patients who had participated in another research study involving an investigational product in the prior 12 weeks or had gene or cellular therapy at any time prior to this study were excluded.

TABLE 1. Demographic and Genetic Characteristics of 6 Male Patients With Choroideremia Treated With Gene Therapy

Patient No.	Age (Years)	Race	Ethnicity	*CHM* Genotype	
501	50	White	Hispanic	Substitution	Arg450Met AG(G)>AT(G)
502	53	White	Non-Hispanic	Null	c.525_526delAG
503	49	White	Non-Hispanic	Null	Arg239Stop CGA>TGA
504	72	White	Non-Hispanic	Null	c.525_526delAG
505	50	White	Non-Hispanic	Null	Thr175del2acAG
506	32	White	Non-Hispanic	Null	p.Arg267Ter (R267X)

• **CLINICAL ASSESSMENTS:** Clinical assessments were performed during screening, 2 days (day -2) prior to the surgery date (day 0), and at days 1 and 7 and months 1, 3, 6, 9, 12, 18, and 24 after surgery. At each visit, patients received a full ophthalmic examination, including ETDRS BCVA, intraocular pressure, slit-lamp, lens opacity, and dilated ophthalmoscopy assessment. FAF and SD-OCT (Spectralis; Heidelberg Engineering, Franklin, Massachusetts, USA) were assessed at screening and at all postsurgical visits. Microperimetry (MAIA; Centervue, Fremont, California, USA) was performed at screening and at each visit starting at 7 days after surgery. Visual fields were evaluated using Goldmann perimetry at screening and at 12 and 24 months. Contrast sensitivity assessment using Pelli-Robson chart and color vision assessment using Roth 28 Hue test were performed at screening and at each visit starting at 1 month after surgery. For study analysis, the FAF, microperimetry, SD-OCT, and fundus photography were assessed in a masked manner by the Doheny Image Reading Center (Los Angeles, California, USA). The FAF measurement included the area of foveal autofluorescence, which reflects the area of retinal pigment epithelium (RPE) preservation. The SD-OCT measures included the OCT measure subfoveal choroidal thickness and the area of the ellipsoid zone or the photoreceptor inner segment/outer segment band, which represents the junction between inner segment and outer segment, or the inner segment ellipsoid, that is, the distal-most portion of the inner segment.

• **STATISTICAL ANALYSIS:** The change in ETDRS letters from baseline in the treated eye was compared with the change from baseline in the untreated eye at each time point. The change from baseline of the area of autofluorescence was computed at each time point for each eye. For categorical/binary data, the number and proportion of patients pertaining to each category were calculated with 95% confidence intervals (CI). For continuous data, the mean, 95% CI, and standard deviation (SD) were calculated. Given the small sample size, the study was not

TABLE 2. Adverse Events of 6 Male Patients With Choroideremia Treated With Gene Therapy

Adverse Events[a]	Number of Subjects (Subject No.) (N = 6)	Severity	Start Posttreatment	End Posttreatment
Conjunctiva hemorrhage, edema	6 (501, 502, 503, 504, 505, 506)	Mild	Day 1	Day 26 to day 28
Subretinal fluid	5 (501, 502, 503, 504, 506)	Mild	Day 1	4 on day 6 1 on day 26
Extrafoveal macular retinal hole in area of nonfunctioning retina[b]	2 (502, 503)	Mild	Day 1	Not resolved
Anterior chamber cells	1 (506)	Mild	Day 1	Day 7
Vitreous cells	1 (501)	Mild	Day 1	Day 6
Diplopia	1 (505)	Mild	Day 15	Day 36
Cataract[c]	1 (504)	Moderate	Day 89	Day 228 (cataract surgery)

[a]All adverse events occurred in treated eye.
[b]In each case, a small retinal hole developed in identifiable partial-thickness thinning defect visible on preoperative optical coherence tomography.
[c]Worsening of preexisting cataract; patient aged 72 at enrollment.

TABLE 3. Subjective Visual Observations of Treated Eye After Gene Therapy of 6 Male Patients With Choroideremia

	Patient No.						
Subjective Visual Observation in Treated Eye	501	502	503	504	505	506	Total Subjects
Vision "clearer" or "sharper"		x	x		x	x	4
"Mild shade"	x		x	x			3
"Sees color better"		x				x	2
"Light sensitivity" or "glare"	x			x			2
"Better contrast"		x					1
"Vision brighter"						x	1
"Sees stars better"				x			1
"Night vision a little better"						x	1
"Increased visual noise"			x				1

Observations cited by each patient are indicated by an "x."

designed with sufficient power to support hypothesis testing.

RESULTS

• **PATIENT DEMOGRAPHICS AND GENOTYPES:** The age, race, ethnicity, and disease-causing genotypes of the 6 subjects with choroideremia are summarized in Table 1. Age at enrollment ranged from 32 to 72 years. One patient had a *CHM* genotype resulting in an amino acid substitution of the REP1 protein, and the other 5 patients had predicted *CHM* null genotypes with premature termination of REP1 translation leading to nonsense-mediated decay.

• **SAFETY EVALUATIONS:** All adverse events occurred in the treated eyes and are summarized in Table 2. No serious adverse events occurred. Most of the adverse events were expectedly and likely attributable to vitrectomy with subretinal fluid injection surgery, including conjunctival hemorrhage, anterior chamber cells, vitreous cells, subretinal fluid, and cataract. Patient 504, aged 72 years at enrollment, had worsening of preexisting cataract requiring cataract surgery 7 months after gene therapy. For Patients 502 and 503, each developed a macular retinal hole in an area of nonfunctioning retina where baseline OCT demonstrated preexisting lamellar thinning. Patient 505 developed transient diplopia from postoperative week 2 to week 5.

• **IMMUNOLOGIC RESPONSE:** Results of viral shedding by PCR amplification of vector genomes in blood, tears, saliva, and urine at screening and at day 1, day 7, month 1, month 3, and month 6 were all below the detectable level of 50.0 copies/5 μL, except for Patient 501 on day

TABLE 4. Best-Corrected Visual Acuity of 6 Male Patients With Choroideremia Treated With Gene Therapy

Patient No.	Visual Acuity Baseline (ETDRS Letter Score)		Visual Acuity Month 12 (ETDRS Letter Score)		Visual Acuity Month 24 (ETDRS Letter Score)		Change in Letter Score, Baseline to Month 24		Change in Letter Score, Treated Eye vs Untreated Eye, Month 24
	Treated Eye	Untreated Eye	Treated Eye	Untreated Eye	Treated Eye	Untreated Eye	Treated Eye	Untreated Eye	
501	65	81	69	79	70	80	5	−1	6
502	61	77	71	78	71	77	10	0	10
503	56	69	54	71	56	71	0	2	−2
504	58	77	58	75	57	75	−1	−2	1
505	75	78	78	84	77	82	2	4	−2
506	77	80	80	80	79	80	2	0	2

1, with 136.5 copies/5 μL in tears of the treated eye, and Patient 502 on day 1, with 64.3 copies/5 μL in saliva. Results of ELISA assay of serum anti-AAV-2 neutralizing antibody responses were all < 10 for 5 patients (Patients 502-506) at screening and at day 1, day 7, month 1, month 3, and month 6. Patient 501 had anti-AAV-2 neutralizing antibody of 451 (screening), 400 (day 1), 450 (day 7), >19 200 (month 1), 17 360 (month 3), and 19 200 (month 6). ELISPOT assay of T cell–mediated immune responses showed negative responses (<3-fold compared to negative control and ≥ 50 spot forming units/10^6 cells) to AAV-REP1 and REP1 peptides in all patients for posttreatment samples. Only mild anterior chamber cells and vitreous cells were observed in all patients, typical of inflammatory response seen after standard vitrectomy.

• **SUBJECTIVE VISUAL OBSERVATIONS:** Subjective visual observations from the treated eyes of patients at follow-up visits during the 24-month period after gene therapy are summarized in Table 3. Observations considered favorable include "clearer" or "sharper" vision, "sees color better," "better contrast," "sees stars better," "vision brighter," and "night vision a little better." Observations considered neutral or possibly unfavorable included "light sensitivity" or "glare," "mild shade," and "increased visual noise."

• **VISUAL ACUITY:** Table 4 shows the following data: (1) the ETDRS scores at baseline, month 12, and month 24; (2) changes in letter score from baseline to month 24; and (3) changes in letter scores of treated eye vs untreated eye at month 24. At baseline, the BCVA scores of the treated eyes ranged from 56 letters (~20/80) to 77 letters (~20/30), and the BCVA scores of the untreated eye ranged from 69 letters (~20/40) to 81 letters (~20/25). At month 24, the changes in letter scores from baseline ranged from -1 to +10 letters for the treated eyes and from -2 to +4 letters for the untreated eyes. The changes in letter scores of treated eyes vs untreated eyes at 24 months ranged from -2 to +10 letters.

Figure 1 shows the change in BCVA scores from the baseline of the study visits. The treated eye of Patient 502 showed a gain of 10 letters at month 1 that was sustained through month 24. Patient 504 was aged 72 years at enrollment and had worsening of preexisting cataract in the treated eye that necessitated cataract surgery at month 7, and the BCVA showed a corresponding drop at month 3 and month 6. After the cataract surgery, the BCVA of the treated eye returned to baseline BCVA at month 9.

The mean changes in BCVA from baseline to month 24 in treated and untreated eyes are shown in Figure 2. A modest improvement of mean change in BCVA in the treated eyes was primarily driven by the BCVA improvements in Patients 502 and 501, while the mean change in BCVA in the untreated eyes remained unchanged at month 24.

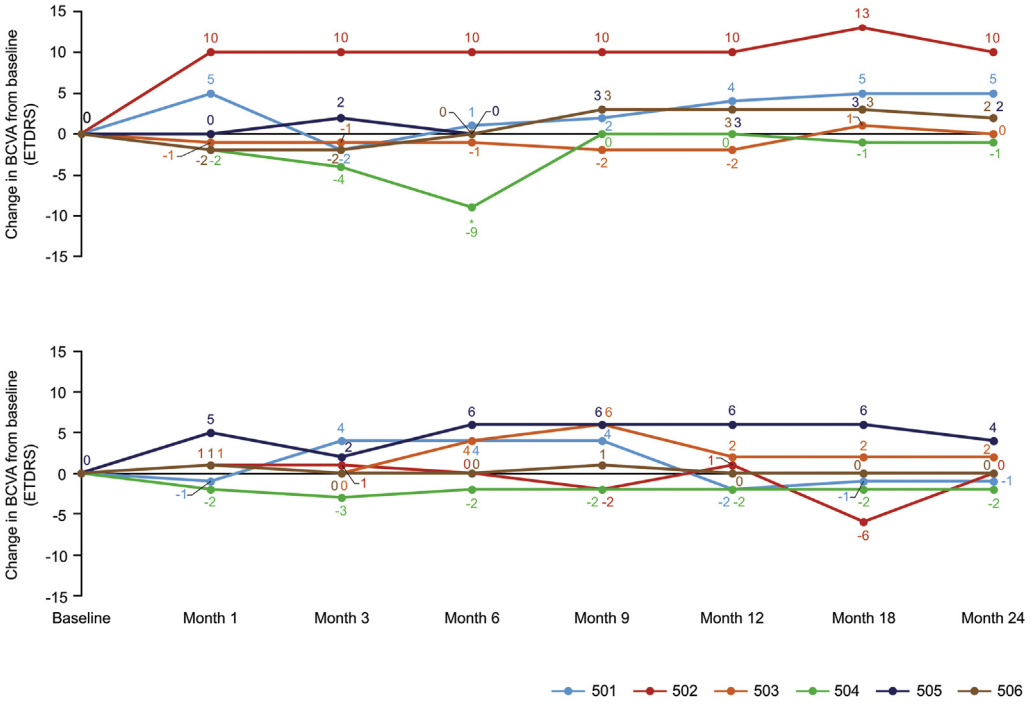

FIGURE 1. Change in Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) from baseline to month 24 in the treated (Top) and untreated (Bottom) eyes of each patient. Patient 504 had worsening of preexisting cataract in the treatment eye after gene therapy surgery and had cataract surgery between the 6-month and 9-month visits.

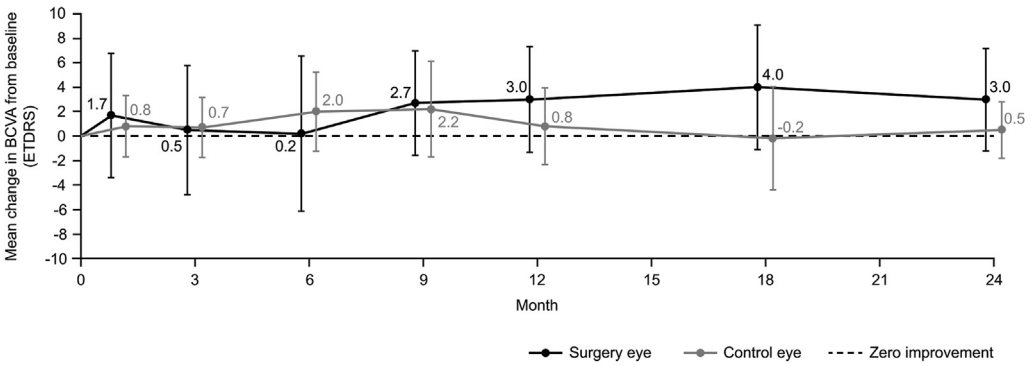

FIGURE 2. Mean change in best-corrected visual acuity (BCVA) from baseline to month 24 in treated and untreated eyes. ETDRS = Early Treatment Diabetic Retinopathy Study.

• **MICROPERIMETRY, VISUAL FIELD, COLOR VISION, CONTRAST SENSITIVITY:** The microperimetry results are shown in Table 5. The very low average thresholds at baseline are typical of patients with advanced choroideremia. No substantial microperimetric changes are noted at month 24, and no notable changes in visual field, color vision, and contrast sensitivity are found between baseline and after treatment. Patient 502, who had a BCVA gain of 10 letters in the treated eye, had improved average threshold in both the treated and untreated eye.

• **ANATOMIC ENDPOINTS:** Given that the patients had advanced choroideremia with small areas of retained retina at the fovea, the area of foveal autofluorescence at baseline ranged from 0.4 to 11.8 mm^2 in the treated eyes vs 0.5 to 11.9 mm^2 in the untreated eyes. Both eyes exhibited shrinkage in autofluorescence over follow-up. There was no difference between treated and untreated eye shrinkage at either month 12 or month 24. The area of SD-OCT ellipsoid zone at baseline exceeded scan size and could not be measured in Patients 501 and 506 and for the remaining patients ranged from 0.5 to 2.9 mm^2 in the

TABLE 5. Microperimetry Data of 6 Male Patients With Choroideremia Treated With Gene Therapy

	Microperimetry (Average Threshold dB)					
	Baseline		Month 24		Change	
Patient No.	Treated Eye	Untreated Eye	Treated Eye	Untreated Eye	Treated Eye	Untreated Eye
501	1.9	4.2	0.8	3.8	−1.1	−0.4
502	0	1.2	4.1	6.6	+4.1	+5.4
503	0	0.4	0	0	0	−0.4
504	0	0	0.2	0.3	+0.2	+0.3
505	0	0.5	0	0.5	0	0
506	5.1	6.1	5.7	6	+0.6	−0.1

treated eyes vs 1.3 to 2.4 mm^2 in the untreated eyes. Too few measurements were available for meaningful statistical analysis; however, there is no evident pattern to the shrinkage observed, other than that shrinkage occurred. The subfoveal choroidal thickness at baseline ranged from 0 to 241 μm in the treated eyes vs 46 to 249 μm in the untreated eyes. With the exception of 1 eye, there was either no change in choroidal thickness or some very modest thinning; however, Patient 506 exhibited thickening in the treated eye from month 1 through month 18 and thinning in the untreated eye.

DISCUSSION

THE 24-MONTH RESULTS OF THIS PHASE 2 GENE THERAPY clinical trial in male patients with advanced choroideremia show that sustained improvement or maintenance of BCVA is achievable with subfoveal high-dose AAV2-REP1 treatment. In addition, the study provides data highly supportive of a favorable safety profile, relating to both the vector and the subretinal route of administration using an automated foot pedal–controlled system for injecting the vector slowly under real-time visualization with MIOCT guidance. Taken together, the safety and efficacy outcomes of this study offer valuable positive contributive evidence in the planning of future clinical trials in patients with choroideremia.

Mean change in BCVA from baseline to month 24 was numerically improved in the treated eyes and stable in the untreated eyes (Figure 2), and this was driven by the BCVA improvement of +10 letters in Patient 502 and +5 letters in Patient 501 (Figure 1, Table 4). The BCVA of the treated eyes of the other 4 patients were stable and ranged from -1 to +2 letters at month 24. All patients had foveal dysfunction from choroideremia, with baseline BCVA of the treated eyes ranging from 56 letters (~20/80) to 77 letters (~20/30). This allowed the safety and efficacy assessments of the subfoveal high-dose AAV2-REP1 treatment in eyes ranging from mild to moderate BCVA impairments. The 10-letter improvement in the treated eye of Patient 502 was achieved at month 1 and sustained to the end of the study period at month 24. This improvement is consistent with BCVA improvements that occur in some patients with low-dose AAV2-REP1 treatments, previously reported by MacLaren and associates and by Edwards and associates.[1,15] One proposed hypothesis for this gain in vision is that it could be owing to the reversal of cellular function, back to near normal, as a result of the clearance of toxic intracellular debris by the introduction of the REP1-producing *CHM* gene. Consistent with this hypothesis of reversal of function, the treated eye of Patient 502 improved by 10 letters despite having the least foveal autofluorescence area and OCT ellipsoid zone area of all treated eyes at baseline. Patient 504, aged 72 years, had decreased BCVA in the treated eye at month 3 and month 6 from worsening of preexisting cataract. The BCVA returned to baseline BCVA after cataract surgery at month 9 after gene therapy. Similar to the MacLaren study[1] but in contrast to the Alberta study,[16] we noted no significant BCVA improvement over time in any of the untreated eyes, indicating that improvement in BCVA could be used as a viable primary outcome for future choroideremia gene therapy trials for patients with advanced choroideremia.

Of interest, given the very low averaged microperimetric thresholds of treated and untreated eyes at baseline, which is typical of patients with advanced choroideremia, no notable changes in microperimetry thresholds were seen at month 24 (Table 5). The patients' subjective visual observations of the treated eye were favorable overall (Table 3) and were important for judging the results, as there is no vision-oriented quality-of-life questionnaire validated for choroideremia patients.

The safety evaluations of this clinical trial are highly supportive of a favorable and sustained safety profile of subfoveal high-dose AAV2-REP1 treatment. No serious adverse events occurred, and most of the adverse events were typical of those related with vitrectomy and subretinal fluid injection surgery, including worsening of existing cataract in Patient 504, which led to cataract surgery

(Table 2). The most worrisome complications were macular retinal hole formation in Patients 502 and 503, although this had no visual effect, as the involved retina area was already nonfunctioning based on baseline OCT. Preoperative OCT should be helpful in predicting surgical risk related to preexisting lamellar retinal hole, especially when retinal thinning is present in areas of functioning retina. Intraoperative OCT helps to monitor progressive retinal thinning with risk of macular hole formation and to determine need to slow or stop the subretinal injection.

The immunologic safety results were favorable. Detectable viral shedding by PCR amplification of vector genomes in blood, tears, saliva, and urine were found only in Patient 501 day 1 tears of the treated eye and Patient 502 day 1 saliva. Patient 501 had significantly increased serum anti-AAV-2 neutralizing antibody after treatment that peaked at month 1, but the only clinical evidence of ocular inflammation was mild vitreous cells that resolved by day 6, which is not unusual after vitreoretinal surgery. All patients had negative posttreatment T cell–mediated immune responses to AAV-REP1 and REP1 peptides.

The surgical procedure with submacular delivery of the AAV-REP1 viral vector appeared to be safe in these patients with advanced retinal atrophy. Intravitreal injection of diluted intravitreal triamcinolone permitted visualization and removal of the posterior hyaloid, which was noted to be thin and poorly adherent but required careful manual elevation by peeling rather than by aspiration. In contrast, the retina was thin but markedly adherent to the underlying choroid and sclera, particularly in areas of dense atrophy but also in the perifoveal target zones. The initial elevation of the retina with BSS was felt to be essential for entering the subretinal space and distinguishing it from the sub-RPE or suprachoroidal space. This 2-step technique (injection of BSS followed by the viral vector) allowed coverage of the predetermined treatment target zone and minimized wasting the viral vector during the attempt to penetrate and lift the tightly adherent retina.

Confirmation of subretinal rather than suprachoroidal injection is difficult with direct observation through the microscope owing to highly altered retina, RPE, and choroid. Intraoperative use of MIOCT allowed real-time visualization of the retina layers, confirmation of the correct tissue plane, and avoidance of sub-RPE or suprachoroidal delivery of BSS or the viral vector.[17] In addition, careful observation of the fovea with real-time intraoperative OCT during the injection of BSS and the vector helped avoid excessive stretching of the foveal tissue and reduced the risk of macular hole. Stretching was also avoided by delivering the subretinal injections in multiple small portions, allowing the subretinal fluid to reabsorb slightly between applications. Careful preparation of the BSS and vector syringes without turbulence or trapped air reduced the risk of subretinal injection of air bubbles. Irrigation and aspiration of the vitreous cavity at the end of the surgery was intended to remove any refluxed vector from the vitreous cavity and may have contributed to the favorable immunologic profile after surgery. Attention to surgical details is likely important for safety.

The FAF and SD-OCT analyses were limited owing to the the small, nonrandomized sample size and the relatively short follow-up. Both eyes exhibited shrinkage in foveal autofluorescence (AF) over follow-up and there was no difference between treated and untreated eye shrinkage. The loss of AF in choroideremia occurs from the edge of the remaining RPE tissue.[20] The current study targeted the central remaining RPE rather than the entire area of viable retina. After foveal detachment with saline (usually from the superior aspect), there was no attempt to continue the subretinal injection and risk stretch-related damage to the fovea. Instead, vector was administered in a second step as soon as the fovea was seen to have lifted on the intraoperative OCT, which limits treatment to the superior and central retina. The half-life of the AF area is 5.0–6.1 years. Over 2 years there would be a predicted loss of AF area of 20%–24%, equivalent to a loss of 11%–13% in the linear dimension.[20] Detaching and transducing at least 90% of the surviving AF and ellipsoid zones on the horizontal plane would be needed to reach the edge that is likely to be lost within 2 years, and which may have already entered a state of irreversible degeneration. Longer follow-up will be needed to see if there is a treatment effect that slows retinal degeneration. Treating patients in earlier stages of disease, before the development of choroidal adhesions, might allow detachment of the entire central retina and 100% exposure to vector in the viable zone.

Limitations of this study include the small, nonrandomizing sample size, given that it is a phase 2 open-label clinical trial of a rare disease. The study lacks a control group other than the fellow untreated eyes of the subjects. Asymmetry of the natural progression of disease between the treated and untreated eyes could confound results. This can only be addressed through randomization of patients to treatment or nontreatment in larger phase 3 studies.

In conclusion, the study provides evidence that treatment of choroideremia with high-dose subfoveal gene therapy using 10^{11} gp AAV2-REP1 has the potential to maintain BCVA, as well as improve BCVA in some cases, indicating that improvement in BCVA could be used as a viable primary outcome for future choroideremia gene therapy trials for patients with advanced choroideremia. Choroideremia gene therapy safety is enhanced with automated injection guided by real-time MIOCT. Larger-scale studies are required to ascertain the significance of these initially encouraging results.

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