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GENE THERAPY FOR LEBER HEREDITARY OPTIC NEUROPATHY: LOW AND MEDIUM DOSE VISUAL RESULTS

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Abstract

OBJECTIVE—To determine the effects of escalating doses of AAV2(Y444,500,703F)-P1ND4v2 in patients with LHON caused by the G11778A mutation in mitochondrial DNA.

DESIGN—Prospective open-label, unilateral single-dose, intravitreal injection of AAV2(Y444,500,703F)-P1ND4v2 per subject in a phase I clinical trial study initiated in 2014.

PARTICIPANTS—Fourteen patients with visual loss and mutated G11778A mitochondrial DNA.

INTERVENTION—Intravitreal injection with the gene therapy vector AAV2(Y444,500,703F)-P1ND4v2 into one eye. Six participants with chronic bilateral visual loss greater than 12 months (Group I), 6 participants with bilateral visual loss less than 12 months (Group II) and 2 participants with unilateral visual loss (Group III) were treated. Nine patients had at least 12 months of follow-up. Clinical testing included ETDRS visual acuity, visual fields, OCT, PERG and neuro-ophthalmic examinations. GEE methods were used for longitudinal analyses.

PRIMARY OUTCOME MEASURE—Loss of visual acuity.

RESULTS—For Groups I and II, month 12 average acuity improvements with treatment relative to baseline were 0.24 logMAR. Fellow eyes had a 0.09 logMAR improvement. A post-hoc comparison found that at month 12, the difference between study eye minus fellow eye improvement in Group II patients of 0.53 logMAR was greater than that observed in our prior acute natural history patients of 0.21 logMAR ($p=0.053$). At month 18, the difference between study eye minus fellow eye improvement in our acute Group II gene therapy patients of 0.96 was greater than that observed in our prior acute natural history patients (0.17 logMAR) $p<0.001$. Two patients developed asymptomatic uveitis that resolved without treatment. OCT of treated eyes had

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Dr. Guy is an inventor of the technology used in this Study (US Patent 7,405,284 "Reducing cellular dysfunction caused by mitochondrial genes) and it is possible that he may receive royalties in the future should the product ever become commercialized.

Here we initiated a human gene therapy trial in the USA designed to determine the effects of escalating doses of a modified adenoassociated vector containing a normal nuclear-encoded NADH:ubiquinone oxidoreductase subunit 4 (ND4) gene.

an average temporal RNFL thickness of 54 μm prior to injection and 55 μm at month 12. For fellow eyes prior to injection it was 56 μm dropping to 50 μm at month 12, $p = 0.013$. GEE suggested that PERG amplitudes worsened more in treated eyes than in fellow eyes by about 0.05 μV (p exchangeable = 0.009). No difference between eyes in outcomes of other visual function measures was evident.

CONCLUSIONS—Allotopic gene therapy for LHON at low and medium doses appears safe and does not damage the temporal RNFL opening the door for testing of the high dose next.

Introduction

Leber Hereditary Optic Neuropathy (LHON) was first associated with a G to A transition at nt-11778 in the ND4 subunit gene of complex I of mtDNA that changes an arginine to histidine at amino acid 340.¹ Visual loss is usually severe and bilateral. Unilateral visual loss is typically followed by fellow eye involvement within months, but intervals greater than a year² and even as long as 18 years³ have been described. A prospective study of the natural history of visual acuity found that spontaneous recovery of visual acuity of 3 lines or more occurs in ~18% of patients.⁴ Two other primary mutations G3460A and T14484C have also been identified in LHON patients' mtDNA that are each associated with a higher rate of spontaneous recovery.^{5;6}

Here, we recoded the ND4 subunit of complex I in the “universal” genetic code and imported into the mitochondrion from the cytoplasm by adding a targeting sequence derived from the P1 isoform of subunit c of ATP synthase (ATPc).⁷ We refer to the nuclear encoded ND4 with the appended ATPc targeting sequences as *P1ND4v2*. It was inserted into a self-complementary adenoassociated vector scAAV2(Y444,500,730F). We first determined its safety and tolerability in preclinical studies of rodents and nonhuman primates,⁸ before testing in our first 5 LHON patients.⁹ Here we describe our findings with longer-term follow-up of these patients in addition to 9 more patients enrolled since that publication for a total of 14 participants.

Participants and Methods

Study Entry and Dosing

The study is approved by the University of Miami Institutional Review Board, FDA, RAC of the NIH and monitored by a DSMC constituted by the NEI. After obtaining informed consent, 14 LHON participants with the G11778A mutation were entered into the study (Table 1). Ten participants were men and four participants were women. All patients were Caucasian except for one who was of partial American Indian heritage. Each received a single intravitreal injection of scAAV2(Y444,500,730F)-*P1ND4v2* in one eye. Six participants had chronic bilateral visual loss longer than 1 year (Group I). The first three received the low dose (5xe⁹ vg) and the next 3 received the medium dose (2.46xe¹⁰vg). Six additional participants had acute bilateral visual loss less than 1 year (Group II). The first three received the low dose study drug and the next 3 received the medium dose. Two participants with unilateral visual loss received the low dose into the eye with good vision before it lost central vision. As of November 2016, these participants have returned for 5 or

more post injection visits (3 to 18 months) and nine patients had at least 12 months of follow-up. The short term visual results of the first 3 patients (participants 1–3) with chronic visual loss treated with the low dose, one (participant 4) with chronic visual loss treated with the medium dose and one patient with acute visual loss treated with the low dose (participant 7) were previously reported.¹⁰

Group I

The bilateral chronic group includes participants with ≥12 months since onset of visual loss in one eye and at least 6 months onset in the more recently affected eye. For inclusion in the study, both eyes must have acuity reduced to ≤35 letters (Snellen = 20/200). If both eyes have ≥12 months since onset, the eye with worse visual acuity is injected. If both eyes have the same acuity, the eye with longest duration of vision loss is injected. If one eye has <12 months and >6 months since onset and the difference in acuity between the eyes is ≤10 letters, the eye with ≥12 months is injected. If eyes have an acuity difference >10 letters, the eye with worse acuity is injected.

Group II

The acute group includes those with bilateral loss of visual acuity to ≤35 ETDRS letters in both eyes for less than 12 months. The worse eye is injected.

Group III

The acute unilateral group includes those with loss of acuity to ≤35 ETDRS letters in one eye, with mildly impaired but good acuity ≥70 letters (Snellen = 20/40) in the contralateral eye. The better eye is injected.

The primary outcome measure is a loss of visual acuity to no light perception for Groups I and II but for Group III a 3 line drop in acuity of the injected eye relative to the fellow eye vision recorded at baseline. Entry into the Study included two baseline visits, followed by the injection, clinic visits on post injection day 1, 7, 30, 60, 90, 180, 270, 365 and then visits every 180 days for up to 3 years. Serial testing included best-corrected visual acuity using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart performed by a certified ophthalmic technologist masked to the identity of the treated eye. Qualifying visual acuity was assessed at a baseline 1 visit, while change in acuity during follow-up was determined from a pre-injection baseline 2 measurement to guard against regression to the mean. Humphrey 30-2 white-on-white standard visual field, pattern electroretinogram (PERG) and spectral-domain optical coherence tomography (SD-OCT) were also performed. Fixation during the visual field is monitored by the study technician. (For the OCT and fundus photos the photographer insures that images are taken of the optic nerve head. For the PERG, the operator controls from the side that the subject orients gaze towards the stimulus display. As the PERG response is dominated by RGCs that have their highest density around the fovea, the large PERG stimulus (25 deg) covers central RGCs even with moderate eccentric fixation. Fixation eccentricities within 5 degrees do not significantly alter the PERG signal.) Neuro-ophthalmic examination included assessments of the pupils, anterior segment and ophthalmoscopy of the optic nerve head and retina.

Molecular Analysis

A PCR-based test using the amplification refractory mutation system (ARMS) was used to detect the presence or absence of 11778 G>A mutation as previously described.⁹

Optical Coherence Tomography

Peripapillary retinal nerve fiber layer (RNFL) thickness was measured as previously described.⁹ OCT scans were only collected at baseline, day 7, day 60, month 12, and annually thereafter.

Pattern Electroretinogram

The PERG was simultaneously recorded from both eyes according to a paradigm optimized for glaucoma detection (PERGLA).^{11;12}

Study Drug and Dose Preparation

Study drug was manufactured by the University of Florida, Powell Gene Therapy Center in their vector core laboratory in January 2013 and stored in 200 µL aliquots in a -80 freezer until use. Stability of the study drug was assessed yearly from January 2014 to January 2016 and no changes were found since manufacture.

Intravitreal injection procedure

Participants were injected in an outpatient clinic room as previously described.⁹

Neutralizing antibody assay

The neutralizing antibody (NAb) titer was reported as the highest serum or anterior chamber sample dilution that inhibited self-complementary AAV2(Y444,500,730F)- smCBA-*mCherry* transduction by 50% compared with no serum control.⁹

Quantitative SYBR-Green qPCR

Quantitative SYBR-Green PCR was performed using the bGH poly A primers (bovine growth hormone polyadenylation signal): F1-5'- AGCCTCGACTGTGCCTTCTAGTT-3' and R1-5'-GGGTTCTGCTATTGTCTTCCCAA with SsoAdvanced™ universal SYBR® Green Supermix (Cat. 172-5271) according to a standard protocol as previously described.⁹

Statistical Analysis

For each visual function parameter, logMAR acuity, HVF MD, RNFL thickness, and PERG amplitudes, improvement from baseline in injected eyes was compared to untreated fellow eyes with the paired t-test. The first baseline measurement of acuity was used to establish patient eligibility and the second baseline was used to calculate improvement to guard against regression to the mean. Longitudinal analysis of changes was performed with GEE. We used both exchangeable and 1st order autoregressive correlation structures and these gave similar results.

This phase I study was not randomized and did not include an uninjected control group. Therefore, we compared changes from baseline to those observed in our previously

published natural history cohort⁴. For these analyses we restricted inclusion to those natural history patients who would have been eligible for this gene therapy trial at baseline. We assigned the worse eye of natural history patients as the study eye to act as a surrogate for the treated eye in the current study and the better eye as the fellow eye. When both eyes of eligible natural history patients had identical baseline acuities, we randomly selected the study eye. Of note, visits of our natural history patients occurred every six months, limiting the follow-up times that could be directly compared to the gene therapy patients. Changes from baseline in the study eyes versus the fellow eyes were compared between the two patient cohorts stratifying by onset group with two-factor analysis of variance.

Results

VISUAL ACUITY

Groups I-II—We plotted the change in Logarithm of the Minimum Angle of Resolution (logMAR) visual acuity of injected and fellow eyes by patient visit for Group I (Fig. 1) and Group II (Fig. 2). A difference of 0.1 logMAR is equivalent to a change of 1 line or 5 letters on the ETDRS chart. Table 2 show the logMAR values of Groups I and II. In Group I one patient started to improve 7–30 days post injection and maintained improvements out to month 18. As no treated eye of Group I lost acuity we treated the next cohort, those with acute bilateral visual loss. Four patients in Group II improved within a month of treatment and continued to improve or maintain improvements with longer follow-up.

Comparison of injected to fellow eyes of gene therapy patients—We attempted to ascertain the significance of changes in acuity of treated eyes, first by analysis relative to fellow eyes that received no treatment as the controls. Comparisons of logMAR acuity of the injected eye minus the fellow eye changes of Groups I and II (low and medium doses combined) revealed that after the day of injection (D01) injected eye acuities were never worse than fellow eye acuities, Table 3. We also compared acuity changes in treated and untreated eyes, each relative to pretreatment at the baseline 2 visit. For treated eyes this ranged from 0.19 logMAR to 0.45 logMAR from post injection day 07 to month 18. For untreated eyes differences between baseline 2 and follow-up examinations were much smaller than seen with treatment ranging from 0.08 logMAR at day 7 to 0.13 logMAR at month 18. Differences in treated eyes relative to the baseline 2 visit (Table 3) reached statistical significance at days 07, 30, 60, 90 and at months 6 and 18. When improvement of all injected eyes of Groups I and II was compared to the fellow eyes using all measurements from day one post injection (D01) through month 24 (M24), the injected eye improvement was significantly greater than the fellow eye improvement ($p=0.051$, GEE exchangeable correlation structure; $p=0.087$, GEE 1st order autoregressive correlation structure). However, in combining the acute and chronic cohorts the size of the difference in injected eye improvements was 4 letters.

Comparison of injected eyes in this gene therapy study to untreated patients prospectively entered into a natural history study - Next, we used a control group of untreated patients followed in a prior natural history study⁴ that we had designed to determine in prospective fashion the course of visual function in untreated G11778A LHON, including only those

patients who would have been eligible for the current study at their baseline visits (n=24 chronic and n=9 acute), stratifying by chronic versus acute onset. For the natural history study patients, we used the worse eye at baseline as a surrogate for injected eyes. A post-hoc comparison found that the difference between study eye minus fellow eye improvement in our acute onset bilateral visual loss gene therapy trial patients of 0.53 logMAR was greater than that observed in our prior acute onset natural history patients (0.21 logMAR units), ($p=0.053$) at month 12. At month 18 the difference between study eye minus fellow eye improvement in our acute onset bilateral visual loss gene therapy trial patients of 0.96 logMAR was greater than that observed in our prior acute onset natural history patients (0.17 logMAR units), ($p<0.001$). In that natural history study, among acute eyes worse at baseline, we found that at 12 months follow-up 44% of eyes had spontaneous improvement of visual acuity of 3 lines or more. In this gene therapy study 66% of treated eyes improved by 3 lines or more. In the natural history study, 22% of the acute onset eyes worse at baseline lost 3 lines of acuity, while in this gene therapy study, no injected eyes (Groups I and II) lost 3 lines of acuity.

Group III—As none of the treated eyes in Groups I and II showed worsening of acuity we next tested whether treatment in the good eye (70 letters (20/40) or better) of those with unilateral visual loss of would prevent the deterioration characteristic of LHON with profound visual loss in both eyes. One of the two patients in group III lost vision 3 months after injection from a baseline of 85 to 60 letters in the treated eye. At 6 months it dropped further to 5 letters (see Table 1). However, acuity of the fellow eye was equivalent with 5 letters at baseline and also at 6 months post injection. The second patient maintains good vision with 74 letters at 6 months relative to 77 letters at baseline.

VISUAL FIELDS

For Group II at 12 months follow-up (n=3) treated eyes slightly improved from -26.9 dB to -21.6 dB while fellow eyes worsened from -21.7 dB to -23.4 dB. Overall, for Groups I and II, we did not find any noteworthy changes with treatment relative to the baseline 2 visit or any differences between treated eye and fellow eye changes at any time point except at 90 days post injection ($p = 0.047$) (Table 4). GEE did not find anything significant (p exchangeable = 0.143, p autoregressive = 0.145). At 12 months follow-up there was little difference in the chronic cohort between our gene therapy patients (average treated eye minus fellow eye improvement = -0.64) and our prior natural history group (average treated eye minus fellow eye improvement = 2.15). However, in the acute bilaterally affected cohort there was a large difference between gene therapy patients (average treated eye minus fellow eye improvement = 7.01 dB) and the natural history group (average treated eye minus fellow eye improvement = -3.95 dB). Much of this difference was due to worsening of the fellow eyes of gene therapy patients rather than improvement of the treated eyes. This might be interpreted as a treatment induced stabilization of the ongoing visual field degeneration.

SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY (SD-OCT)

Average retinal nerve fiber layer (RNFL)—Serial measurements of the overall average retinal nerve fiber layer (RNFL) are tabulated in Table 5. As an intravitreal injection of AAV2 is expressed mainly in the macula of nonhuman primates we looked at the temporal

quadrant of the optic nerve head that subserves macular fibers. Average temporal RNFL of Groups I and II (n=9) combined was thicker in treated than in fellow eyes at month 12, $p = 0.013$. In eyes selected for treatment average temporal RNFL thickness at the baseline 2 visit was 54 μm and at month 12 it was 55.0 μm . In fellow eyes on the day prior to injection (BL2), average temporal RNFL measurements were 56 μm and dropped to 50 μm at month 12. This suggests, treatment preserved the temporal RNFL subserving macular fibers targeted by an intravitreal AAV2 injection.

PATTERN ELECTRORETINOGRAM (PERG)

Pattern electroretinogram amplitudes at baseline were extremely low in Group I, Group and even in Group III eyes with good vision (Table 6). Normal amplitude is approximately 1.0 μV . Amplitudes remained low at the post treatment follow-up examinations. GEE suggested that PERG amplitudes worsened more in the treated eyes than the fellow eyes (p exchangeable = 0.009, p autoregressive = 0.011) by about .05 uV. With comparison to the PERGs obtained in our natural history study there were no significant main effects or interactions (all $p > 0.56$). We wondered whether the injection procedure had an adverse effect on the PERG. Comparisons of the PERG on day 1 post injection relative to baseline showed a mean drop of 0.08 μV in treated eyes, $p = 0.035$. In fellow eyes average day 1 post injection PERG was unchanged from baseline ($p = 0.97$). Thus, the drop in PERG amplitude appears to be related to the injection since expression from the vector would not have initiated this quickly.

ADVERSE EVENTS

All adverse events are listed in Table 7. Two of 14 patients (14%) experienced asymptomatic transient mild anterior uveitis (Fig. 3). In both cases uveitis was detected at the 2 month post injection visits. One patient was in Group I and treated with the medium dose, while the other patient was in Group II and treated with low-dose study vector. In both cases uveitis cleared spontaneously without treatment. One of 14 patients lost vision in the treated eye. This was detected at the 3 month post injection visit. This patient was in Group III and received treatment in an eye with 20/16 vision. At 6 months visual acuity of treated and untreated eyes was the same at 5 letters in each eye. Other adverse events included transient post injection spike of intraocular pressure, painful toxic/exposure keratitis, subconjunctival hemorrhage, sore throat and headaches.

NEUTRALIZING ANTIBODIES (Nabs) AND QPCR

Neutralizing antibodies to AAV2 were detected in all participants prior to injection (Table 8). Strong inhibition of the red fluorescence of mCherry in cultured cells indicating the presence of high levels of NAb against AAV2 (titer of 20,480) were found in serum samples of 4/14 (28.5%) LHON subjects at baseline, day 1, day 7, month 3, month 6 and month 12 post injection. Seven of fourteen patients showed low Nabs at baseline. Of the treated LHON patients who developed anterior uveitis, in one who received a medium dose the levels of serum neutralizing antibodies rose from a titer of 80 at baseline to a titer of 20,480 at day 7 and remained unchanged at 3 and 12 months post injection. In the other patient levels of neutralizing antibodies were extremely high at baseline (titer of 20,480) and remained at these levels. One other patient with an NAb titer of 5 (received medium dose

vector) at baseline and post injection (day 1 and day 7) showed a transient rise of to a titer of 20 at 3 months post injection. Two other subjects had moderate NAb levels (titers of 5120 and 320) at baseline, day 1, day 7 and month 3. Nabs in the anterior chamber fluid of all participants were much lower than the serum levels. Blood samples were negative for vector DNA (Table 9) indicating no leakage of the injected rAAV into the systemic circulation.

Discussion

While our phase I study was initially designed to evaluate safety rather than efficacy, when we compared treated eyes to fellow eyes of all patients in Groups I and II we did find a statistically significant effect of treatment on visual acuity improvement. The French Gensight study group in a press release (<http://www.businesswire.com/news/home/20161219006275/en/GenSight-Biologics-Reports-Sustained-Visual-Acuity-Gain>) also described a beneficial effect of allotropic ND4 gene therapy in their 14 patients of 0.61 logMAR with 18 months of follow-up. At baseline, their treated eyes were hand movements that they ascribed to a value of 2.79 logMAR. So in their low vision logMAR schema an improvement from HM to CF is 0.8 logMAR, whereas for our trial it is 0.3 logMAR. There are inconsistencies among gene therapy studies in the assignment of logMAR values to poor vision. The same investigative group assigned HM acuity as logMAR=2.0 in one publication¹³, but in another publication¹⁴ used logMAR=3.0 for HM. We followed Holladay's¹⁵ convention of CF=2.0 logMAR, but felt that assigning Holladay's logMAR score of 3.0 to HM (equivalent to a 10 lines worse than CF) would weight changes off the ETDRS chart too heavily, swamping losses and improvements of patients with acuities on the chart. We therefore assigned a conservative but meaningful difference of 0.3 logMAR (equivalent to 3 lines) worse to HM (2.3) and a further 0.3 logMAR worse to LP (2.6).

In our trial study drug improved visual acuity by 3 lines or more in one of six participants with visual loss longer than 12 months. The gene therapy clinical trial in China reported improvements of 3 lines or more in 5 of 9 G11778A LHON patients (7 of their patients were treated more than one year after visual loss) with 36 months of follow-up.^{16;17} In our natural history study,⁴ only 3 patients recovered vision of 3 lines or more 12 months after the onset of visual loss (7%). In our gene therapy study we observed increases in acuity of approximately 3 lines or more on the ETDRS chart in 3 of 6 patients treated within a year of visual loss. In the Chinese study 2 patients were treated within one year of visual loss, one showing improvement while the other did not.^{16;17}

In designing the study we altered the timing of treatment to those with visual bilateral loss for more than a year (group I), less than a year (group II) and those who still retained good vision in one eye (group III). With the low and medium doses thus far, we have seen improvements in group I (n = 1 of 6 patients) and group II (n = 3 of 6 patients) and have seen loss of vision in group 3 (n = 1 of 2). At this point the response to treatment seems to be best when given with a year of visual loss. Improvements in vision of treated eyes was seen quickly within 7–30 days post injection, during a time when expression in preclinical studies was seen in 90% of rodent RGCs.¹⁸ Vision in these patients improved further with longer follow-up. Rapid and persistent expression of the vector seen in our animal studies may have played some role.¹⁹ While our rodent studies have demonstrated that transgene

expression and integration of the allotopic ND4 into complex I of the rodent retina occurs in almost all RGCs within one week, it is unlikely that similar experiments will be done to determine whether this is also the case in human gene therapy patients as their eyes are not available for histochemical studies.

Despite vision improvements the PERG analysis suggests that injection of this vector has a small adverse effect on retinal ganglion cell function. In our preclinical animal studies we did find a drop in the PERG amplitude with injection of recombinant AAV containing an irrelevant gene (green fluorescent protein or cherry) into control eyes.^{20;21} It is unlikely that human gene therapy trials will include sham intravitreal injections into control eyes to ascertain effects on the PERG. Although a non-mitochondrial retinal disease, LCA2 caused by recessive mutations in the RPE gene *RPE65* has undergone long term follow up in patients after subretinal AAV vector mediated delivery of the normal RPE65 cDNA.²²

Outcomes from 14 participants enrolled in this trial treated with the low and medium dose study vector found no serious safety concerns associated with allotopic gene therapy for LHON. One patient in Group III lost vision due to the course of bilateral visual loss characteristic of LHON that was unaltered by treatment with the low dose. We judged the primary adverse event definitely related to study drug was anterior uveitis. Fortunately, uveitis associated with our gene therapy was mild, asymptomatic, transient and required no treatment. It is unclear whether it was related to levels of serum neutralizing antibodies. One of the patients had a rise in serum neutralizing antibodies post injection while the other had no change in neutralizing antibodies that were at the highest levels detected in our study prior to injection. While uveitis was not seen in our natural history of LHON patients,⁹ ocular inflammation was seen in our preclinical work with nonhuman primates and it also resolved without treatment or sequelae as detected clinically and at necropsy.²³ The Chinese gene therapy trial for G11778A LHON did not describe uveitis in any of their 9 treated patients. They had medicated their patients with oral prednisone one week before treatment and continued it for 2 months after intravitreal injections.²⁴ Ocular events related to the study drug injection procedure were elevated intraocular pressure and toxic keratitis in a single patient that we previously reported.⁹ Pre-and post-injection blood samples were negative for vector DNA, indicating there was no significant leakage of vector into the systemic circulation.

Despite the absence of vector DNA in participant blood samples, neutralizing serum antibodies (Nabs) to AAV2 that could potentially reduce the expression of the delivered wild-type *ND4* gene or cause ocular inflammation were elevated in all participants prior to injection and increased in 3 participants. Uveitis was associated with a rise in serum neutralizing antibodies in one patient but not in the other who had very high levels at baseline. Surprisingly the four patients that showed the most improvement in visual acuity with treatment had the highest levels of serum neutralizing antibodies. Baseline Nab levels detected from intraocular fluid removed at the time of injection were much lower than serum levels in all participants. The 4 patients with improved vision had similar low levels of Nabs, detected in ocular fluid, as those that did not recover visual acuity. Taken together these findings suggest that high serum levels of Nabs may not be a barrier to successful ocular gene therapy.

In contrast to our initial preclinical studies showing rescue of oxidative phosphorylation in G11778A LHON cells,⁷ Oca-Cossio and coworkers²⁵ reported that allotopic expression killed cultured cells, thus was deemed unsafe. Here we showed that the temporal RNFL of our treated patients followed for 12 months was not damaged by allotopic ND4 gene therapy. One year post treatment values were similar to those obtained prior to treatment. In contrast, temporal RNFL of fellow eyes continued to thin. If this finding was due to a lack of reproducibility of OCT in our patients it would lead to attenuated correlations and insufficient statistical power, that is, an inability to find truly significant effects. Thus, we can see how lack of reproducibility could explain our failure to find an effect for average RNFL, but not the difference we noted between loss rates at the temporal quadrant in injected versus fellow eyes.²⁶ This finding could be spurious and confirmation would be desirable, but lack of reproducibility is not the cause. Thus, our OCT data shows here that allotopic ND4 was not harmful to visual function and it did not damage the temporal RNFL subserving macular fibers targeted by an intravitreal AAV2 injection.^{27;28} These results pave the way for injection of high dose cohorts next.

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Figure 1.

Plot of changes in visual acuity (logMAR) of injected and uninjected fellow eyes by visit of participant 1 (A), participant 2 (B), participant 3 (C), participant 4 (D) participant 5 (E) and participant 6 (F). Participants 1,2 and 3 were treated with low-dose study drug and participants 4, 5 and 6 were treated with the medium dose, all had chronic visual loss for more than one year.

Figure 2.

Plot of changes in visual acuity (logMAR) of injected and uninjected fellow eyes by visit of participant 7 (A), participant 8 (B), participant 9 (C), participant 10 (D), participant 11 (E) and participant 12 (F). Participants 7, 8 and 9 were treated with low-dose study drug and participants 10, 11 and 12 were treated with the medium dose, all had acute visual loss for less than one year.

Figure 3.

The eye of a patient treated with the low-dose (A) shows fine keratic precipitates (B). A second patient treated with the medium dose shows keratic precipitates (C) that resolved one month later (D).

Table 1

Patient Characteristics

Group	Dose	Injection Number	Gender	Age	Ethnicity	Months since initial acuity loss	Study Eye			Fellow	Study Eye last visit ETDRS letters	Fellow Eye Last visit ETDRS letters	Last Visit
I. Chronic	Low	1	M	45	W	56	OS	09/2014	HM	HM	CF	CF	Mo 24
		2	M	56	W	251	OD	11/2014	CF	CF	CF	CF	Mo 24
		3	M	37	W	26	OS	01/2015	14	18	10	12	Mo 18
		4	M	33	W	16	OD	04/2015	HM	HM	1	4	Mo 18
	Medium	6	M	29	W	138	OD	06/2015	11	11	13	13	Mo 18
		8	M	24	W	13	OD	07/2015	CF	CF	CF	CF	Mo 12
		5	F	49	W	9	OD	04/2015	15	20	53	39	Mo 18
		7	M	22	W	5	OS	06/2015	LP	CF	17	HM	Mo 18
II. Acute	Low	9	M	23	W	6	OD	09/2015	4	6	17	9	Mo 12
		10	F	26	Am Ind	8	OS	12/2015	CF	CF	1	1	Mo 06
	Medium	11	F	27	W	7	OS	02/2016	2	5	6	6	Mo 06
		13	F	31	W	2	OS	04/2016	13	15	7	13	Mo 03
III. Unilateral	Low	12	M	19	W	5	OS	03/2016	90	4	5	5	Mo 06
		14	M	52	W	8	OS	06/2016	77	4	74	63	Mo 06

W = white race; Am Ind = American Indian; BL2 = Measurement made at second baseline visit; Mo = Follow up month

2018, November 01.

Table 2

Summary of logMAR acuity for groups I and II

		Mean (SD) Log MAR Acuity [†]			
	Visit	N _C , N _A	Injected Eye	Fellow Eye	Difference
Pre-injection	BL2	12 6, 6	1.85 (0.41)	1.78 (0.37)	0.08 (0.17) p [*] =0.14
	Day 1	12 6, 6	1.80 (0.39)	1.71 (0.33)	0.09 (0.21) p=0.16
	Day 7	12 6, 6	1.66 (0.34)	1.69 (0.40)	-0.03 (0.29) p=0.72
Post-injection follow up	Day 30	12 6, 6	1.65 (0.36)	1.70 (0.32)	-0.05 (0.19) p=0.37
	Day 60	12 6, 6	1.65 (0.35)	1.69 (0.38)	-0.03 (0.10) p=0.27
	Day 90	12 6, 6	1.69 (0.38)	1.69 (0.37)	0.00 (0.08) p=1.00
	Mo 6	11 6, 5	1.73 (0.36)	1.75 (0.34)	-0.03 (0.07) p=0.22
	Mo 9	8 6, 2 ^{††}	1.67 (0.39)	1.67 (0.39)	0.00 (0.08) p=0.87
	Mo 12	9 6, 3	1.66 (0.38)	1.72 (0.38)	-0.06 (0.25) p=0.48
	Mo 18	7 5, 2	1.47 (0.42)	1.68 (0.46)	-0.21 (0.25) p=0.17

Mean (SD) Log MAR Acuity [‡]				
	Visit	N _C , N _A	Injected Eye	Fellow Eye
	Mo 24	2	2.00 (0.00)	2.00 (0.00)
		2, 0		
				Difference 0.00 (0.00) p=1.00

N_C = Number of chronic (group I patients), low and medium doses; N_A = Number of Acute (group II) patients, low and medium doses; BL2 = baseline 2; Mo = Month

[‡] Equivalent low vision logMAR acuities: CF = -2.0, HM = -2.3, LP = -2.6

* 2-tailed paired t-test p values assess significance of average difference between injected and fellow eye

^{††} 2-1-08 missed their 9 month study visit but returned for their 12 month visit

Table 3

Summary of logMAR acuity improvements from baseline 2 for groups I and II

		Mean (SD) Log MAR Acuity Improvement [†]		
Visit	N _C , N _A	Injected	Fellow	Difference
Day 1	12 6, 6	0.05 (0.1) p [*] =0.10	0.07 (0.15) p=0.16	-0.02 (0.11) p=0.65
Day 7	12 6, 6	0.19 (0.23) p=0.015	0.08 (0.18) p=0.15	0.11 (0.20) p=0.091
Day 30	12 6, 6	0.20 (0.24) p=0.015	0.08 (0.12) p=0.043	0.13 (0.24) p=0.095
Day 60	12 6, 6	0.20 (0.23) p=0.012	0.09 (0.23) p=0.20	0.11 (0.26) p=0.17
Day 90	12 6, 4	0.16 (0.24) p=0.037	0.09 (0.26) p=0.28	0.08 (0.19) p=0.19
Mo 6	11 6, 5	0.16 (0.22) p=0.038	0.06 (0.27) p=0.49	0.11 (0.18) p=0.085
Mo 9	8 6, 2 ^{††}	0.15 (0.28) p=0.19	0.12 (0.26) p=0.26	0.03 (0.10) p= 0.39
Mo 12	9 6, 3	0.24 (0.37) p=0.081	0.09 (0.28) p=0.36	0.15 (0.43) p=0.32
Mo 18	7 5, 2	0.45 (0.46) p=0.039	0.13 (0.34) p=0.34	0.32 (0.57) p= 0.18
Mo 24	2 2, 0	0.15 (0.21) p=0.50	0.15 (0.21) p=0.50	0 (0) p=1.00

N_C = Number of chronic (group I patients), low and medium doses; N_A = Number of Acute (group II) patients, low and medium doses; Mo = Month

[†] Equivalent low vision logMAR acuities: CF = -2.0, HM = -2.3, LP = -2.6

^{*} 2-tailed paired t-test p values assess significance of average difference between follow up and baseline 2 measurement

^{††} SAA missed the 9 month visit, but returned for their 12 month visit

Table 4

Summary of HVF Mean Deviation for groups I and II

		Mean (SD) HVF MD			
	Visit	N _{NC} , N _A	Injected	Fellow	Difference
Pre-injection	BL2	12 6, 6	-26.6 (7.7)	-23 (10.7)	-3.7 (6.6) p=.078
	Day 1	12 6, 6	-24.0 (9.6)	-24.1 (7.6)	0.0 (5.0) p=.982
	Day 7	12 6, 6	-26.2 (7.6)	-22.9 (8.8)	-3.4 (3.8) p=.010
Post-injection follow up	Day 30	12 6, 6	-26.5 (9.0)	-25.0 (8.2)	-1.5 (1.9) p=.019
	Day 60	12 6, 6	-27.2 (8.0)	-26.7 (8.3)	-0.5 (1.4) p=.214
	Day 90	12 6, 6	-25.1 (7.6)	-25.6 (6.7)	0.5 (2.3) p=.486
	Mo 6	11 6, 5	-25.1 (8.4)	-24.8 (8.7)	-0.3 (3.3) p=.737
	Mo 9	8 6, 2 ^{††}	-24.5 (12.0)	-24.2 (9.8)	-0.3 (4.9) p=.859
	Mo 12	9 6, 3	-24.6 (11.8)	-24.4 (10.6)	-0.2 (3.1) p=.820
	Mo 18	7 5, 2	-22.7 (12.4)	-22.0 (11.0)	-0.7 (5.7) p=.077
	Mo 24	2	-28.1 (6.5)	-25.0 (10.6)	-3.1 (4.1)

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Mean (SD) HVF MD				
Visit	N N _C , N _A	Injected	Fellow	Difference p=0.48
	2, 0			

N_C = Number of chronic (group I patients), low and medium doses; N_A = Number of Acute (group II) patients, low and medium doses; BL2 = baseline 2; Mo = Month

* 2-tailed paired t-test p values assess significance of average difference between injected and fellow eye
†† 2-1-08 missed their 9 month study visit but returned for their 12 month visit

RNFL - Measurements of Participants with 1 year of follow-up sorted by order of injected eye logMAR improvement from baseline 2 to month 12

Table 5

Injection Number	Injected Eye				Fellow Eye				
	logMAR Improvement	RNFL thickness (µm)			logMAR Improvement	RNFL thickness (µm)			
		Temporal		Average		Temporal		Average	
		BL2	M12	M12		BL2	M12	BL2	M12
3	-0.10	58	55	61	62	-0.18	54	54	65
2	0.00	50	55	51	53	0.00	46	49	50
1	0.00	53	54	54	55	0.30	56	57	58
8	0.00	53	56	57	52	0.00	59	54	61
6	0.04	51	51	54	57	0.08	47	45	62
9	0.26	50	57	100	61	0.06	60	44	117
5	0.32	65	55	103	75	0.18	48	49	104
4	0.74	56	61	62	56	0.68	70	52	80
7	0.94	50	51	101	58	-0.30	62	47	143
Mean (SD)	0.24 (0.37)	54.0 (5.0)	55.0 (3.0)	71.4 (22.7)	58.8 (6.9)	0.09 (0.28)	55.8 (7.9)	50.1 (4.4)	82.2 (31.9)

BL2 = baseline 2; M12 = month 12

Table 6

PERG Amplitude (uV), all groups

Injection Number	Eye	Baseline		Post injection follow up											
				Day						Month					
		1	2	1	7	30	60	90		6	9	12	18	24	
1	Inj	0.17	.18	.27	.05	.27	.26	N/A		.24	.19	.22	.18	N/A	
	FE	0.30	.24	.25	.11	.37	.31	N/A		.20	.16	.30	.20	N/A	
2	Inj	0.45	.48	.40	.57	.46	.30	.30		.33	.44	.33	.16	.36	
	FE	0.35	.42	.46	.40	.49	.31	.33		.33	.43	.30	.17	.25	
3	Inj	0.16	.45	.25	.24	.33	.23	.35		.32	.55	.35	.16		
	FE	0.38	.32	.44	.27	.42	.34	.33		.32	.38	.44	.19		
4	Inj	0.25	.10	.08	.13	.09	.27	.28		.10	.14	.25	.11		
	FE	0.13	.16	.08	.14	.14	.19	.24		.08	.34	.13	.39		
6	Inj	0.32	.34	.29	.46	.37	.32	.41		.17	.23	.24	.32		
	FE	0.48	.21	.40	.44	.42	.33	.32		.23	.20	.25	.48		
8	Inj	0.27	.38	.36	.33	.47	.46	.39		.33	.27	.30			
	FE	0.43	.41	.39	.22	.47	.44	.47		.25	.43	.27			
5	Inj	0.19	.23	.06	.14	.12	.16	.22		.20	.13	.15	.13		
	FE	0.23	.16	.31	.22	.25	.15	.17		.17	.15	.19	.20		
7	Inj	0.31	.42	.07	.37	.37	.47	.31		.42	N/A	.25	.47		
	FE	0.41	.40	.14	.38	.18	.44	.28		.38	N/A	.40	.33		
9	Inj	0.56	.58	.52	.56	.56	.56	.38		.36	.41	.56			
	FE	0.54	.56	.37	.54	.54	.52	.69		.55	.29	.54			
10	Inj	0.25	.23	.28	.30	.27	.33	.15		.34					
	FE	0.32	.29	.32	.36	.29	.23	.37		.31					
11	Inj	0.20	.36	.20	.32	.19	.18	.18		.30					
	FE	0.29	.28	.30	.39	.29	.21	.17		.24					
13	Inj	0.69	1.04	1.01	.58	.69	.79	.67							
	FE	0.67	.98	.99	.81	.72	.97	.67							
12	Inj	0.41	.39	.30	.28	.24	N/A	.27		.34					

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Injection Number	Eye	Baseline		Post injection follow up											
				Day						Month					
		1	2	1	7	30	60	90	6	9	12	18	24		
	FE	0.32	.27	.17	.36	.22	N/A	.30	.22						
14	Inj	0.34	.22	N/A	.43	.40	.35	.40							
	FE	0.45	.23	N/A	.44	.47	.41	.40							

Inj = injected eye; FE = fellow eye; N/A = not available

Table 7

Adverse Events

Injection Number	Temporal relationship to injection	Description	Status
Ocular · Related to Injection/Investigational Product			
3	29 d	Small asymptomatic vitreous condensation	No treatment, Ongoing
5	Same day	Intraocular pressure >50mmHg, lightheadedness	Resolved same day
5	Same day	Corneal abrasion	Resolved next day
6	2 m	Mild uveitis	No treatment, Resolved spontaneously
9	2 m	Uveitis	No treatment, Resolved spontaneously
Reported by most patients	Same day	Burning sensation after anesthesia wore off	Resolved same or next day
11	18 d	Occasional mild stinging, possibly related to injection	Resolved
14	1 d	Decreased vision in injected eye of unilateral patient	Resolved spontaneously within 7 days with return to pre-injection acuity which is maintained through 6 month follow up
Systemic · Possibly related to Injection/Investigational Product			
4	2 d	Sore throat (possibly due to seasonal allergies)	Resolved by 14 days
10	2 d	Cough and sore throat	Resolved within 10 days But recurred 3m later and are ongoing
9	1.1 m	Elevated ALT	Resolved by month 2
Ocular · Likely Unrelated to Injection/Investigational Product			
2	2.1 m	Small afferent pupillary defect	Resolved within 1 month
7	2.7 m	Irritation	Resolved in 1 day
6	1.6 m	Pain discomfort in both eyes	Resolved in one month
6	2.2 m	Fellow eye red and twitching	Resolved spontaneously
6	2.3 m	Complains of decreased acuity in both eyes. This decrease was not manifest upon study vision testing in which the patient read more letters than at either baseline 1 or baseline 2. Visual field tests	Ongoing
		did not show worsening from baseline	
12	3.4 m	Decreased acuity in injected eye of unilateral patient attributed to natural course of LHON despite injection	Ongoing
Systemic · Likely unrelated to Injection/Investigational Product [†]			
6	3.8 m	Mucocele on MRI	Ongoing
6	9.5 m	Tingling in legs	Ongoing
7	1 d prior	Lightheaded during blood draw	Resolved by time of injection
7	1.3 m	Headaches attributed to enlarged cisterna magna, unchanged from pre-injection MRI	Headaches stopped within 4 months
2	5.2 m	Hospitalization to treat pre-existing parathyroid gland condition	Resolved post treatment

Injection Number	Temporal relationship to injection	Description	Status
4	17.1 m	Headaches, motion sickness	Ongoing
12	5.2 m	Headaches	Resolved within 2 days
12	3 d prior	Occasional bloody nose	Ongoing
13	29 d	Exacerbation of numbness in feet	Ongoing
5	8 m	Mild chest pain	Resolved within 5 weeks

[†] Other systemic unrelated adverse events [ref #] which resolved included: GI upset [8], sprained ankle [19], allergic reaction to energy drink [23], common cold [27], Flu symptoms [31], and rash on face [32]

Table 8

Neutralizing Antibodies (NAb)

Injection Number	Dose	Anterior chamber fluid (ACF)		Serum Sample				
		Injection Day	BSL	Day 7	Day 90	Year 1		
1	Low	118	20,480	20,480	20,480	20,480		
2	Low	10	20,480	20,480	20,480	20,480		
3	Low	5	5	20	5	5		
4	Medium	5	5,120	5,120	1,280	1,280		
6	Medium	5	80	20,480	20,480	20,480		
8	Medium	5	5	5	5	5		
5	Low	5	20,480	20,480	20,480	20,480		
7	Low	5	320	320	320	320		
9	Low	20	20,480	20,480	20,480			
10	Medium	5	5	5	5			
11	Medium	5	5	5	20			
13	Medium	5	5	5	5			
13	Low	5	5	5	5			
14	Low	5	5	5				

Table 9**Blood qPCR Detection of Vector DNA**

Injection Number	Dose	Visit	qPCR Run	Spike – DNA	Copy number of scAAV2P1ND4 in 0.5 µg blood DNA				Score [†]
					Rep 1	Rep 2	Rep 3	Avg	
1	Low	BL1	1	1.96E+06	0	0	0	0	0
			2	4.98E+05	0	0	0	0	0
		D01	1	2.27E+05	0	0	0	0	0
			2	4.85E+05	0	0	0	0	0
		D07	1	9.47E+05	0	0	0	0	0
			2	3.28E+05	0	0	0	0	0
2	Low	BL1	1	1.79E+06	44	78	0	41	0
			2	3.42E+05	0	0	0	0	0
		D01	1	1.54E+05	20	14	0	12	0
			2	6.72E+05	0	0	0	0	0
		D07	1	2.01E+05	0	0	0	0	0
			2	5.94E+05	0	0	0	0	0
3	Low	BL1	1	1.79E+06	32	28	0	20	0
			2	3.42E+05	0	0	0	0	0
		D01	1	1.54E+05	50	33	0	28	0
			2	6.72E+05	0	0	0	0	0
		D07	1	2.01E+05	161	351	0	171	1
			2	5.94E+05	0	0	0	0	0
4	Medium	BL1	1	2.33E+05	0	201	48	83	0
			2	2.46E+05	15	0	0	5	0
		D01	1	4.86E+05	0	259	419	226	1
			2	5.91E+05	8	0	0	3	0
		D07	1	3.68E+05	0	273	477	250	1
			2	4.25E+05	10	9	0	7	0
6	Medium	BL1	1	8.35E+05	0	0	0	0	0
			2	6.32E+05	0	0	0	0	0

Injection Number	Dose	Visit	qPCR Run	Spike – DNA	Copy number of scAAV2PIND4 in 0.5 µg blood DNA				Score [†]
					Rep 1	Rep 2	Rep 3	Avg	
8	Medium	D01	1	3.51E+05	0	0	0	0	0
			2	5.03E+05	0	0	0	0	0
		D07	1	4.44E+05	0	0	0	0	0
			2	8.29E+05	0	0	0	0	0
		BL1	1	4.28E+05	64	22	72	52	0
			2	6.32E+05	0	0	0	0	0
		D01	1	3.51E+05	406	298	458	387	1
			2	5.03E+05	0	90	0	30	0
		D07	1	4.44E+05	26	50	44	40	0
			2	8.29E+05	60	0	0	20	0
5	Low	BL1	1	2.33E+06	0	55	0	18	0
			2	2.46E+05	92	63	55	70	0
		D01	1	4.86E+05	0	0	0	0	0
			2	5.91E+05	8	0	170	59	0
		D07	1	3.68E+05	0	0	6	2	0
			2	4.25E+05	12	9	0	7	0
		BL1	1	3.09E+05	0	0	0	0	0
			2	3.61E+05	0	0	0	0	0
		D01	1	6.97E+05	0	0	0	0	0
			2	6.08E+05	0	0	0	0	0
7	Low	D07	1	4.75E+05	152	122	117	130	1
			2	6.69E+05	0	0	0	0	0
		BL1	1	1.18E+05	0	0	0	0	0
			2	3.38E+05	0	0	0	0	0
		D01	1	1.86E+05	0	0	0	0	0
			2	3.70E+05	0	0	0	0	0
		D07	1	1.25E+05	0	0	0	0	0
			2	4.88E+05	0	0	0	0	0

Injection Number	Dose	Visit	qPCR Run	Spike – DNA	Copy number of scAAV2PIND4 in 0.5 µg blood DNA				Score [†]
					Rep 1	Rep 2	Rep 3	Avg	
10	Medium	BL1	1	1.31E+05	0	0	0	0	0
			2	6.35E+05	0	0	0	0	0
		D01	1	7.21E+05	0	0	0	0	0
			2	5.82E+05	0	0	0	0	0
		D07	1	5.48E+05	0	18	52	23	0
			2	1.06E+06	37	54	41	44	0
11	Medium	BL1	1	2.10E+05	20	17	25	21	0
			2	3.07E+05	0	0	0	0	0
		D01	1	5.38E+05	15	22	29	22	0
			2	5.80E+05	0	0	0	0	0
		D07	1	8.57E+05	51	40	31	41	0
			2	2.66E+05	0	0	0	0	0
13	Medium	BL1	1	9.87E+005	11	26	0	13	0
			2	9.09E+005	257	317	263	279	1
		D01	1	3.75E+005	0	0	0	0	0
			2	4.91E+005	0	0	0	0	0
		D07	1	2.05E+005	22	10	0	11	0
			2	4.04E+005	0	0	0	0	0
12	Low	BL1	1	2.64E+05	1	1	1	1	0
			2	3.07E+05	1	4	1	2	0
		D01	1	3.88E+05	2	3	3	3	0
			2	3.78E+05	2	2	2	2	0
		D07	1	3.27E+05	0	1	1	1	0
			2	2.55E+05	1	0	1	1	0
14	Low	BL1	1	4.36E+005	212	219	244	225	1
			2	7.67E+005	353	403	407	388	1
		D01	1	1.19E+005	22	18	270	103	1
			2	4.17E+005	0	0	0	0	0

Injection Number	Dose	Visit	qPCR Run	Spike – DNA	Copy number of scAAV2PIND4 in 0.5 µg blood DNA				Score [‡]
					Rep 1	Rep 2	Rep 3	Avg	
		D07	1	1.77E+005	207	0	103	103	1
			2		0	0	0	0	0

BL1 = baseline 1; D01 = point injection day 1; D07 = post injection day 7; Rep = replicate; Avg = average

Scoring 0=0–99, +1=100–500, +2=501–5,000, +3 = 5,001–50,000, +4 = >50,000 copies/0.5µg

* Blood DNA were used for qPCR from two separately extraction.

NTC 500, Copy number 500 as negative.