Immune Response and Intraocular Inflammation in Patients With Leber Hereditary Optic Neuropathy Treated With Intravitreal Injection of Recombinant Adeno-Associated Virus 2 Carrying the ND4 Gene

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Immune Response and Intraocular Inflammation in Patients With Leber Hereditary Optic Neuropathy Treated With Intravitreal Injection of Recombinant Adeno-Associated Virus 2 Carrying the *ND4* Gene

A Secondary Analysis of a Phase 1/2 Clinical Trial

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IMPORTANCE Intravitreal gene therapy is regarded as generally safe with limited mild adverse events, but its systemic effects remain to be investigated.

OBJECTIVE To examine the association between immune response and intraocular inflammation after ocular gene therapy with recombinant adeno-associated virus 2 carrying the *ND4* gene (rAAV2/2-*ND4*).

DESIGN, SETTING, AND PARTICIPANTS This secondary analysis of an open-label, dose-escalation phase 1/2 randomized clinical trial of rAAV2/2-*ND4* included data from February 13, 2014 (first patient visit), to March 30, 2017 (last patient visit at week 96), the first 2 years after injection. Patients older than 15 years with diagnosed *ND4* Leber hereditary optic neuropathy (LHON) and visual acuity of at least counting fingers were enrolled in 1 of 5 cohorts. Four dose cohorts of 3 patients each were treated sequentially. An extension cohort of 3 patients received the dose of 9×10^{10} viral genomes per eye.

INTERVENTIONS Patients received increasing doses of rAAV2/2-*ND4* (9×10^{9}, 3×10^{10}, 9×10^{10}, and 1.8×10^{11} viral genomes per eye) as a single unilateral intravitreal injection. Patients were monitored for 96 weeks after injection; ocular examinations were performed regularly, and blood samples were collected for immunologic testing.

MAIN OUTCOMES AND MEASURES A composite ocular inflammation score (OIS) was calculated based on grades of anterior chamber cells and flare, vitreous cells, and haze according to the Standardization of Uveitis Nomenclature. The systemic immune response was quantified by enzyme-linked immunospot (cellular immune response), enzyme-linked immunosorbent assay (IgG titers), and luciferase assay (neutralizing antibody [NAb] titers).

RESULTS The present analysis included 15 patients (mean [SD] age, 47.9 [17.2] years; 13 men and 2 women) enrolled in the 5 cohorts of the clinical trial. Thirteen patients experienced intraocular inflammation after rAAV2/2-*ND4* administration. Mild anterior chamber inflammation and vitritis were reported at all doses, and all cases were responsive to treatment. A maximum OIS of 9.5 was observed in a patient with history of idiopathic uveitis. Overall, OIS was not associated with the viral dose administered. No NAbs against AAV2 were detected in aqueous humor before treatment. Two patients tested positive for cellular immune response against AAV2 at baseline and after treatment. Humoral immune response was not apparently associated with the dose administered or with the immune status of patients at baseline. No association was found between OISs and serum NAb titers.

CONCLUSIONS AND RELEVANCE In this study, intravitreal administration of rAAV2/2-*ND4* in patients with LHON was safe and well tolerated. Further investigations may shed light into the local immune response to rAAV2/2-*ND4* as a potential explanation for the observed intraocular inflammation.

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Invited Commentary
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Supplemental content

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Leber hereditary optic neuropathy (LHON) is the most common inherited mitochondrial disease.[1-3] It is characterized by preferential involvement of the retinal ganglion cells of the papillomacular bundle with ensuing optic nerve degeneration and severe bilateral vision loss.[1-3] Leber hereditary optic neuropathy is caused by a point mutation in mitochondrial DNA,[4-6] affecting a subunit of complex I (NADH dehydrogenase), an enzyme of the oxidative phosphorylation pathway.[6-9] The G to A substitution at nucleotide 11778 (G11778A) in the NADH dehydrogenase subunit 4 (*ND4*) gene (OMIM 516003) accounts for most cases of LHON and the most severe manifestations.[4,7]

Therapy for LHON has been restricted to supportive measures rather than cure.[10] Interventions that target mitochondria are limited, and treatment for LHON remains wanting. Idebenone (Raxone), a synthetic analogue of coenzyme Q10, has been used by practitioners in the European Union albeit with controversial efficacy, because results from a phase 3 study were not statistically different from placebo.[11]

The eye is considered to be immune privileged and thus constitutes an optimal organ for gene therapy.[12,13] Leber hereditary optic neuropathy is an ideal disease model for genetic intervention because the causative point mutation essentially affects the retinal ganglion cells.[14] Adeno-associated viruses (AAVs) are currently the most promising transfer vectors for gene delivery to the retina; they are not pathogenic, can infect the retinal cells effectively,[15,16] and have low intrinsic immunogenicity.[17] An AAV2 vector containing the wild-type *ND4* gene can easily be administered intravitreally to patients with LHON carrying the G11778A mutation. Several ongoing trials are evaluating the safety and efficacy of such ocular gene therapies, and as yet no serious adverse events (AEs) related to treatment or procedure have been reported.[18-22] Most humans develop immunity against the capsid of AAV early in life (mainly a humoral response) as a consequence of natural exposure to wild-type AAV.[17,23-25] As such, the host immune response is a relevant factor to monitor after gene therapy because it may relate to both the safety and the efficacy of the treatment.

The recombinant AAV2 vector carrying the *ND4* gene (rAAV2/2-*ND4*) (GS010) encodes the wild-type ND4 protein and has a proof of concept that was successfully demonstrated in a rat model of LHON.[26] GenSight completed a phase 1/2 safety and dose-escalation study that investigated the intravitreal administration of rAAV2/2-*ND4* (GS010) in patients with LHON carrying the G11778A mutation.[27] We report a secondary analysis of immune responses in relation to manifestations of ocular inflammation in patients enrolled in this phase 1/2 trial.

Key Points

Question Is the immune response after intravitreal injection of recombinant adeno-associated virus 2 carrying the *ND4* gene in patients with Leber hereditary optic neuropathy associated with intraocular inflammation?

Finding In this secondary analysis of a clinical trial that included 15 patients, systemic immune responses to recombinant adeno-associated virus 2 were transient and consistent with previous observations reported in the literature. Humoral or cellular immune responses were not associated with adverse events of intraocular inflammation.

Meaning These findings suggest that intravitreal administration of recombinant adeno-associated virus 2 carrying the *ND4* gene in patients with Leber hereditary optic neuropathy is not associated with intraocular inflammatory safety concerns that would preclude further investigations.

Methods

Phase 1/2 Clinical Study Protocol

An ongoing open-label, dose-escalation phase 1/2 trial of rAAV2/2-*ND4* that includes 15 patients with LHON has assessed the safety and tolerability of 4 doses of rAAV2/2-*ND4* (9×10^9, 3×10^{10}, 9×10^{10}, and 1.8×10^{11} viral genomes [vg] per eye) administered by intravitreal injection to patients with LHON carrying the G11778A-*ND4* mutation (NCT02064569). This secondary analysis included data from the first 2 years after injection, from February 13, 2014 (first patient visit), to March 30, 2017 (last patient visit at week 96). The intravitreal injection (180 µL) was given in the eye with the worse visual acuity. Patients did not receive any immunomodulatory therapy before intravitreal injection. Four dose cohorts of 3 patients each were treated sequentially. An extension cohort of 3 patients received the dose of 9×10^{10} vg. Written informed consent was obtained from all patients before enrollment, and all data were deidentified. The study received approval of the French Ethics Committee and adhered to the tenets of the Declaration of Helsinki.[28]

The primary end point of the trial was the assessment of safety and tolerability. Secondary end points included visual function, viral shedding, and humoral response to AAV2. Serum samples were collected for immunomonitoring. Aqueous humor samples were also obtained through paracentesis before injection in the last 8 patients enrolled.

Grading of AEs of Ocular Inflammation

The intensity of AEs was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. The intensity of uveitis was assessed by the investigator (C.V.C.) and categorized as mild, moderate, or severe based on the CTCAE definitions (eTable 1 in the Supplement).

Ocular Inflammation Score

In this post hoc analysis, a composite global ocular inflammation score (OIS) was determined using 4 separate grades according to Standardization of Uveitis Nomenclature classification (eTable 2 in the Supplement): anterior chamber cells, anterior chamber flare, vitreous cells, and vitreous haze.[29]

Quantification of Anti-AAV2 Cellular Immune Response

Peripheral blood mononuclear cells (PBMCs) were isolated and an ex vivo interferon γ enzyme-linked immunospot (ELISpot) assay was performed to measure the lymphocyte proliferative responses to AAV2 antigens. The AAV2 VP-1 capsid peptide library (145 peptides) consisted of 15-mers overlapping by 10 amino acids with the adjacent peptide. This library was divided into 3 pools of 48 or 49 peptides. The PBMCs were seeded overnight on ELISpot precoated plates (MabTech) at 2.5×10^5

cells per well (except for the stimulation with concanavalin A: 2.5×10^4 cells per well) in AIM V medium (Thermo Fisher Scientific). Cells were then incubated in triplicates with AAV2 peptides (2 µg/mL) for 20 hours. Medium that contained 1% dimethyl sulfoxide served as a negative control and concanavalin A (2 µg/mL; Sigma-Aldrich) as a positive control. An additional positive control was run using CEF (MabTech), which contains 23 major histocompatibility class I restricted peptides, representing the T-cell epitopes of 3 viruses common in the human population that bind to a broad range of HLA molecules. Spots were revealed using the interferon γ ELISpot assay kit (MabTech). For each stimulation pool, the number of spots per 1×10^6 PBMCs was normalized by subtracting that of the background (negative control). The values obtained for the 3 pools of AAV2 VP-1 peptides were combined. The cellular immune response was considered to be positive when the number of spots was 50 or greater per 1×10^6 PBMCs and at least 3 times greater than in control treated cells.

Quantification of Anti-AAV2 IgG

Anti-AAV2 IgG antibodies were determined by enzyme-linked immunosorbent assay testing of serum and aqueous humor samples prediluted at 1:50. Microtiter plates were coated with rAAV2/2-*ND4* (50 µL per well; 1×10^9 vg/mL) overnight at 5°C. After washing, plates were blocked with 5% bovine serum albumin that contained buffer during 2 hours at room temperature. Serial dilutions of test samples (1:10 to 1:7290) were added to wells and incubated for 2 hours at 37°C. A pool of normal human serum samples was used as a positive control (standard curve, arbitrary units [AU] per milliliters). A donkey antihuman IgG antibody conjugated to horseradish peroxidase (400 µg/mL, 1:20 000; Interchim) was added for 2 hours at 37°C. Plates were finally incubated with a solution of tetramethyl benzidine for 15 minutes at room temperature, the reaction was stopped with hydrochloride 1N, and optical density was read at 450 nm. With use of a standard curve fitted with a 4-factor sigmoid regression, an IgG concentration was reported for each sample. The concentration retained for a sample was the mean of 2 relevant concentrations of 7 measures. The humoral immune response was considered to be positive when the anti-AAV2 IgG concentration was above 1000×10^3 AU/mL and was increased at least 3-fold compared with baseline.

Quantification of Anti-AAV2 Neutralizing Antibodies

HEK293 cells were plated 18 hours before infection at a density of 5×10^4 cells per well. An rAAV2 expressing luciferase under the control of the cytomegalovirus promoter (8×10^7 vg per well) was incubated at 37°C in 5% carbon dioxide for 1 hour, either alone (uninhibited control) or with serial fold dilutions of human serum samples. Each transduction mix was added onto plated HEK293 cells, in duplicate wells, and incubated for 24 hours at 37°C in 5% carbon dioxide. The fraction of luciferase-positive cells was assessed using the Luciferase Assay System (Promega) and a TriStar LB 941 microplate reader (Berthold Technologies). For each sample, a 4-factor sigmoid regression was calculated between the percentage of inhibition of cell infection and the log of serum dilution factors. The half maximal inhibitory concentration was determined using the intercept at 50% of the regression curve and expressed as a dilution factor. The immune response was considered to be positive when the anti-AAV2 neutralizing antibody (NAb) titer was above 1:1000 and was increased at least a 3-fold compared with baseline.

Statistical Analysis

For each patient, OISs and NAb titers were plotted against each other and individual linear regressions were calculated. A Wilcoxon signed rank test was performed on regression slopes. SAS PROC UNIVARIATE was used to determine whether the null hypothesis (slope = 0) could be rejected (SAS software, version 9.4, SAS Institute Inc). No adjustment was made for multiple analyses. A 2-sided *P* value was generated.

Results

Assessment of Ocular Inflammation After rAAV2/2-*ND4* Administration

Fifteen patients (mean [SD] age, 47.9 [17.2] years; 13 men and 2 women) were enrolled in the 5 cohorts. The AEs of ocular inflammation are reported in **Table 1**. Thirteen of 15 patients experienced intraocular inflammation after rAAV2/2-*ND4* administration. The AEs of inflammation in the anterior chamber and the vitreous were recorded separately. Concomitant anterior and intermediate uveitis were reported in 7 patients.

Table 1. Ocular Inflammation After Intravitreal Injection of Recombinant Adeno-Associated Virus 2 Carrying the *ND4* Gene in Patients With Leber Hereditary Optic Neuropathy[a]

Characteristic	Dose Level				
	9×10^9 vg (n = 3)	3×10^{10} vg (n = 3)	9×10^{10} vg (n = 6)	1.8×10^{11} vg (n = 3)	All (N = 15)[b]
Anterior Chamber Inflammation					
No. (%) of patients	0	2 (20)	6 (60)	2 (20)	10 (100)
No. (%) of events	0	2 (14)	10 (71)	2 (14)	14 (100)
Mild	0	2 (15)	9 (69)	2 (15)	13 (100)
Moderate	0	0	0	0	0
Severe	0	0	1 (100)	0	1 (100)
Vitreous Inflammation					
No. (%) of patients	1 (9)	2 (18)	5 (45)	3 (27)	11 (100)
No. (%) of events	1 (8)	3 (23)	6 (46)	3 (23)	13 (100)
Mild	1 (8)	3 (25)	5 (42)	3 (25)	12 (100)
Moderate	0	0	0	0	0
Severe	0	0	1 (100)	0	1 (100)

Abbreviation: vg, viral genome.

[a] Events were recorded according to the Common Terminology Criteria for Adverse Events.

[b] N = 15 until week 48, then n = 14 until week 96.

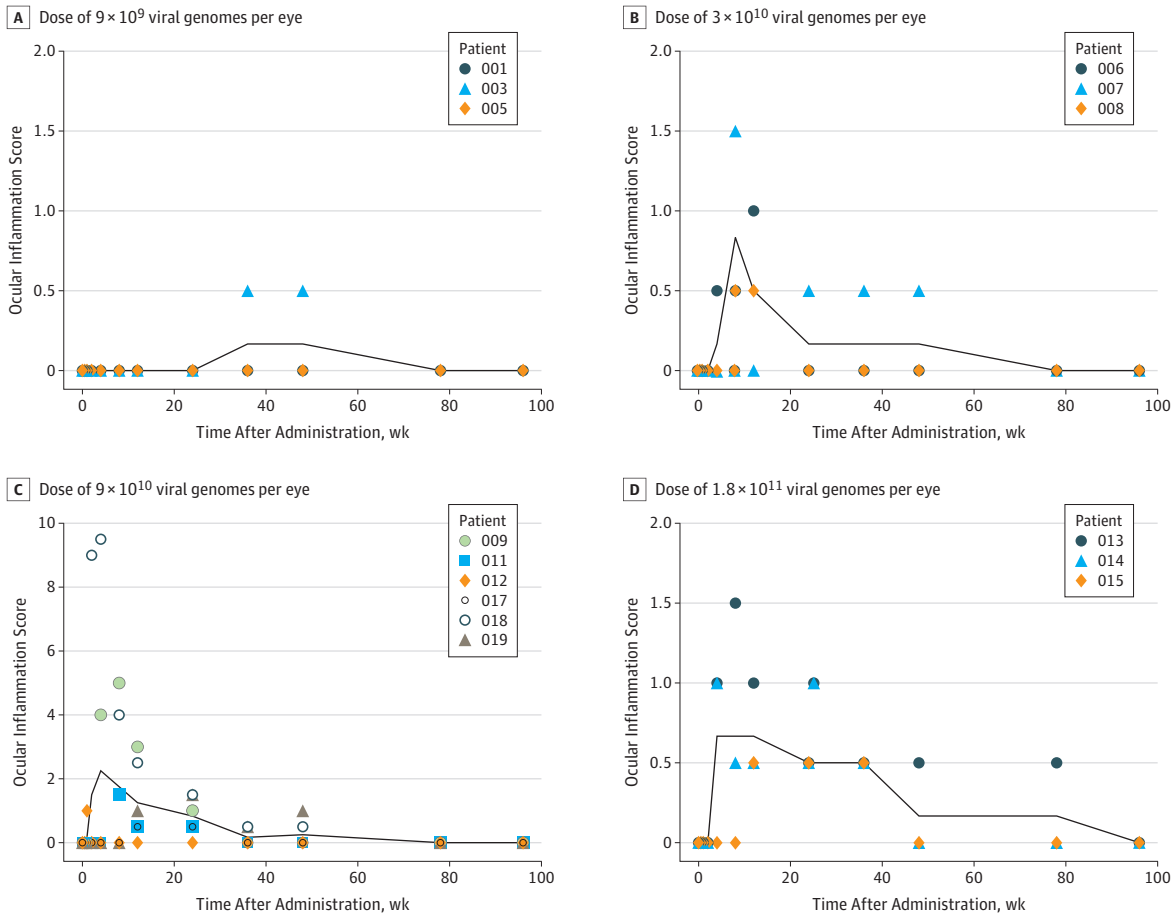
A total of 14 events of anterior chamber inflammation occurred in 10 patients at all dose levels except 9×10^9 vg starting from 7 to 93 days after injection. No association was found between intensity of ocular inflammation and dose administered. All events resolved after treatment with topical anti-inflammatory therapy (eTable 3 in the Supplement). Anterior chamber inflammation was considered by the investigator (C.V.C.) to be probably associated with treatment with rAAV2/2-*ND4* except in 2 cases that involved the untreated eye of a patient with a history of idiopathic uveitis.

Vitritis (intermediate uveitis) was reported at all dose levels and was considered by the investigator (C.V.C.) to be probably related to treatment. A total of 13 events of vitreous inflammation occurred in 11 patients (Table 1). Eight of 13 events were treated with topical anti-inflammatory agents, and 3 events in 2 patients were also treated with oral corticosteroids (eTable 3 in the Supplement). All treated events resolved without sequelae. Four events resolved without treatment (limited, nonevolving presence of cells in the vitreous), and 1 patient with untreated ongoing mild vitritis withdrew consent past 48 weeks and was unavailable for follow-up.

All events of ocular inflammation were mild in intensity according to the CTCAE except in 1 patient in the 9×10^{10} vg dose group who experienced concomitant severe anterior chamber inflammation and vitritis 13 days after injection of rAAV2/2-*ND4*. The patient's condition resolved without sequelae after treatment with topical and oral corticosteroids (Table 1 and eTable 3 in the Supplement).

A total of 22 AEs of ocular inflammation were treated with topical corticosteroids: rimexolone (used 9 times), dexamethasone (used 4 times), dexamethasone (used twice), and fluorometholone (used once) (in some cases, concomitant anterior and intermediate inflammation were treated with the same agent). One patient in the 1.8×10^{11} vg dose group was treated with oral prednisone for mild vitritis and small punctate serous detachment, which also resolved. Another patient in the 9×10^{10} vg dose group was treated with oral prednisone for concomitant severe anterior and intermediate uveitis. No sequelae were reported in any patients as a consequence of these AEs.

Figure 1. Ocular Inflammation in Patients With Leber Hereditary Optic Neuropathy After Treatment With Recombinant Adeno-Associated Virus 2 Carrying the *ND4* Gene



Global ocular inflammation scores are given for the different dose levels. The plain line represents the mean, and data points correspond to individual values.

Table 2. Characteristics of Individual Responses to Recombinant Adeno-Associated Virus 2 Carrying the *ND4* Gene

Dose Level, Patient No.	Maximum, OIS	No. of OI Events	IgG ($\times 1^{-3}$ AU/mL)			NAb (IC_{50})			Cellular Response
			Baseline	Maximum Value	Fold Change	Baseline	Maximum Value	Fold Change[a]	
9×10^9 vg									
001	0	0	7115	7565	1	1 440	3050	2	Negative
003	0.5	1	3520	14 895	4	520	3650	7	Negative
005	0	0	1903	5355	3	360	3400	9	Positive
3×10^{10} vg									
006	1	1	155	10 665	69	23	2200	96	Negative
007	1.5	3	33	336	10	0	40	40	Negative
008	0.5	1	536	2585	5	48	488	10	Negative
9×10^{10} vg									
009	5	2	2110	14 240	7	420	3200	8	Negative
011	1.5	2	13	1148	88	0	120	120	Negative
012	1	3	32	177	6	0	60	60	Negative
017	0.5	2	918	1215	1	228	396	2	Negative
018	9.5	3	10 295	67 240	7	2850	40 000	14	Positive
019	1.5	3	26	78	3	0	24	24	Negative
1.8×10^{11} vg									
013	1.5	2	46	760	17	0	68	68	Negative
014	1	2	4952	18 010	4	630	8400	13	Negative
015	0.5	1	155	1229	8	0	220	220	Negative

Abbreviations: AU, arbitrary unit; IC_{50}, half maximal inhibitory concentration; NAb, neutralizing antibody; OI, ocular inflammation; OIS, ocular inflammation score; vg, viral genome.

[a] For null NAb values at baseline, the fold change was calculated with a value of 1.

Post Hoc Analysis of OIS

A global OIS was calculated for the treated eye at each visit (**Figure 1**). At baseline, the OIS was null in all patients. All doses considered, the mean OIS peaked at 1.1 four weeks after administration. The 2 highest individual OISs were reported in a patient in the 9×10^{10} vg with a score of 5 at week 8 and a patient in the 9×10^{10} vg dose group with a score of 9.5 at week 4. All OISs returned to baseline levels at the latest by week 78 (week 96 for a patient in the 1.8×10^{11} vg dose group). The magnitude of the OIS response did not correlate with the dosage administered.

Although all patients with ocular inflammation had an OIS greater than 0, the score was not consistently associated with the intensity of ocular inflammation assessed by the investigator (C.V.C.) using the CTCAE scale (eTable 1 in the Supplement). For example, a patient in the 9×10^{10} vg dose group had a maximum OIS of 5 as the result of concomitant anterior and intermediate uveitis, both of which were considered by the examiner to be of mild intensity.

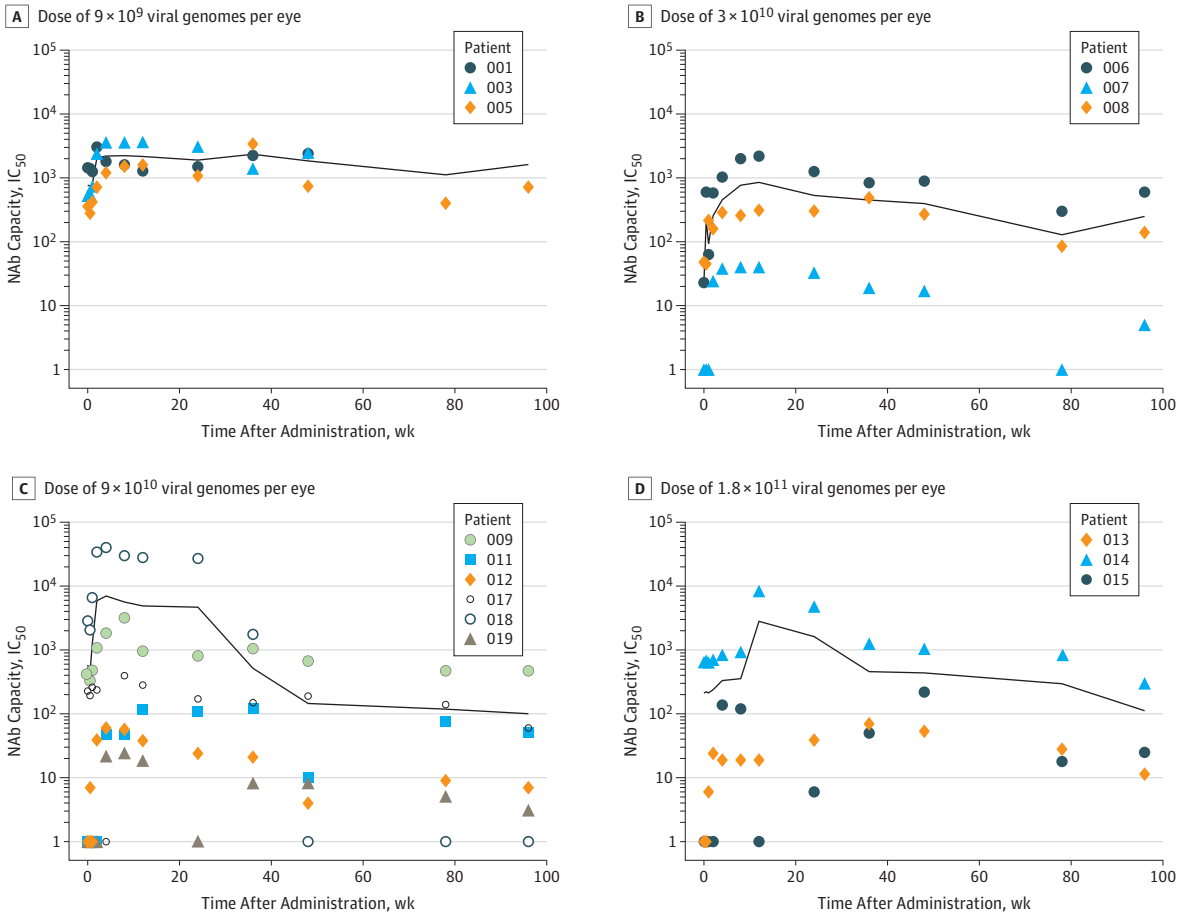
Immunogenicity of rAAV2/2-*ND4* in Patients With LHON

Humoral and cellular immune responses against AAV2 vector were quantified in serum and PBMC samples, respectively, after intravitreal injection of rAAV2/2-*ND4*. Two of 15 patients tested positive for cellular response against AAV2 capsid at baseline (one in the 9×10^9 vg dose group and the other in the 9×10^{10} vg dose group) (eFigure 1 in the Supplement). After injection, the cellular response increased up to 12 times the baseline level in a patient for whom no ocular inflammation was reported. In another patient, the cellular immune response increased up to 6 times the baseline level. No persistent cell-mediated immune response occurred in the other patients; sporadic positivity was observed in 1 patient at week 4 and another at week 8 (eFigure 1 in the Supplement), but this was not confirmed at later time points. No association between cellular response and the viral dose administered was evident.

At baseline, anti-AAV2 IgG levels were highly variable among patients, ranging from 13 to 10 295 $\times 10^{-3}$ AU/mL (with a mean at baseline of 536 $\times 10^{-3}$ AU/mL), with low levels of IgG (<1000 $\times 10^{-3}$ AU/mL) in 9 of the 15 patients (**Table 2**). The overall humoral immune response against AAV2 peaked between 12 and 24 weeks after administration (eFigure 2 in the Supplement), with positive IgG responses in 9 of 15 patients. All IgG levels decreased over time. The anti-AAV2 IgG response did not correlate with levels at baseline or the dose administered. Of note, 1 patient in the 9×10^{10} vg dose group who experienced severe uveitis after rAAV2/2-*ND4* administration presented with a high anti-AAV2 IgG titer at baseline.

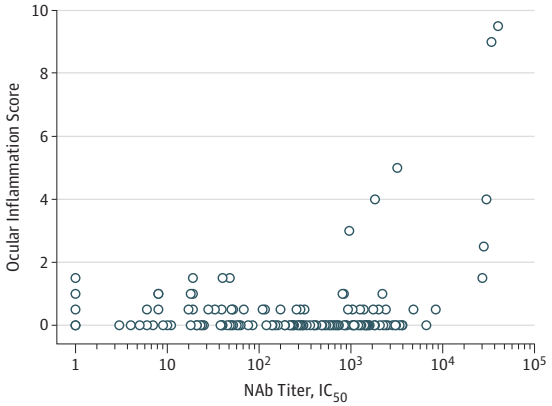
Quantification of anti-AAV2 NAb titers was expressed as the serum dilution factor able to inhibit AAV2 transduction by 50% or more (half maximal inhibitory concentration). Baseline NAb titers were highly variable among patients, ranging from 0 to 1:2850 (median titer, 1:48) in a patient in the 9×10^{10} vg dose group (Table 2). At baseline, anti-AAV2 NAb titers were undetectable in 6 patients, below 1:1000 in 7 patients, and above 1:1000 in 2 patients (1 each in the 9×10^9 vg and 9×10^{10} vg dose groups). A positive NAb response was found in 6 patients, starting 2 weeks after rAAV2/2-*ND4* administration (**Figure 2**). In these patients, NAb titers typically returned to baseline levels at week 36. In a patient in the 9×10^{10} vg dose group, NAbs were undetectable past week 48. Overall, the anti-AAV2 NAb response did not seem to be associated with levels at baseline or the dose administered. For the last 8 enrolled patients, NAbs were also quantified in the aqueous humor before rAAV2/2-*ND4*

Figure 2. Humoral Immune Response in Patients With Leber Hereditary Optic Neuropathy Before and After Treatment With Recombinant Adeno-Associated Virus 2 Carrying the *ND4* Gene



Serum quantification of neutralizing antibodies (NAb) for the different dose levels. The plain line represents the mean, and data points correspond to individual values. IC_{50} indicates half maximal inhibitory concentration.

Figure 3. Relationship Between Ocular Inflammation Score (OIS) and Neutralizing Antibody (NAb) Titer



Plot of individual pairings of OISs and NAb titers for all 15 patients at all time points available. Null NAb titers were assigned a value of 1 to allow for representation on a logarithm scale. IC_{50} indicates half maximal inhibitory concentration.

administration. Samples were negative in all patients, including the patient who had the highest NAb titer in serum at baseline.

Association Between NAb Immune Response and Ocular Inflammation

We hypothesized that the NAb titer at baseline might be associated with the ocular inflammatory response. No association was evident among NAb levels at baseline, the magnitude of the immune response, and the OIS in all 15 patients (Table 2). A patient in the 9×10^9 vg dose group had positive IgG and NAb responses but did not have ocular inflammation. Conversely, 1 patient each in the 3×10^{10} vg, 1.8×10^{11} vg, and 9×10^{10} vg dose groups had no positive immune response but presented with mild ocular inflammation and an OIS of 1.5.

A plot analysis of paired OIS and NAb titers at all time points in all patients did not fit any regression analysis models (**Figure 3**). Null OISs (N = 138) were associated with a range of NAb titers from 0 to 1:6620 (median titer, 1:224). Likewise, null NAb titers (N = 26) were associated with OISs that ranged from 0 to 1.5 (median OIS, 0). A Wilcoxon signed-rank test was run

on individual linear regression slopes and found no association between NAbs titers and OIS.

Discussion

After unilateral intravitreal injection of rAAV2/2-*ND4*, a transient mild increase in serum NAb titer and treatment-responsive uveitis resolved without any long-term sequelae. Occurrence of intraocular inflammation was consistent with a preclinical study[30] using rAAV2/2-*ND4*. Such inflammatory reactions were previously reported in studies[19,21,22,31-34] of ocular gene therapy irrespective of the viral vector (adenovirus and lentiviral vectors), serotype (AAV2 and AAV8), route of administration (intravitreal and subretinal), and administration of prophylactic immunosuppressive therapy. Of note, most clinical trials in ocular gene therapy using peri-injection of immunosuppressive agents reported no intraocular inflammation.[18,34-37] These findings suggest that systematic use of prophylactic corticotherapy should be considered in future trials of rAAV2/2-*ND4* to lessen inflammatory responses.

No association was observed between ocular inflammation and humoral or cellular immune response. Neither ocular inflammation nor immune response could be determined based on the viral dose administered or the patient's immune status at baseline. A patient in the 9×10^{10} vg dose group presented with an atypical clinical profile both at baseline and after injection of a medium dose of rAAV2/2-*ND4*. Before administration, the patient had the highest IgG and NAb titers in serum and tested positive for cellular response against AAV2; 2 weeks after intravitreal injection, the patient had severe ocular inflammation and the highest OIS as well as IgG and NAb titers. In comparison, a patient in the 9×10^{9} vg dose group who had positive IgG, NAb, and cellular responses after injection did not experience any ocular inflammation (OIS, 0), although this finding could be explained by the lowest dose of vector being injected (9×10^{9} vg per eye). In our study, 9 patients received a dose of 9×10^{10} vg per eye or more, and only 1 of these patients presented with severe ocular inflammation.

Our results are consistent with previous work[38] in cynomolgus macaques showing that detection of NAbs in the anterior chamber and serum did not correlate with increased exposure to intravitreal AAV vectors; this finding suggests that immune responses are not specific to the gene therapy product but rather are patient driven. It is known that CpG-rich viral genomes, such as that of the rAAV2/2-*ND4* vector, can activate Toll-like receptors and trigger an innate immune response.[39,40] In our study, not every patient administered a medium or high dose developed an immune response, indicating that rAAV2/2-*ND4* is not highly immunogenic when injected in the vitreous. As expected, no NAbs were detected in the aqueous humor of the patients at baseline regardless of antibody levels in the serum. However, considering that ocular inflammation was reported after vector administration, we cannot confirm that AAV vectors are completely nonimmunogenic or that the eye is an entirely immune-privileged organ. Ongoing animal studies will help determine whether antibodies against rAAV2/2-*ND4* can be detected in the eye after intravitreal administration and shed light on the local immune response to gene therapy.

Limitations

This study is limited by the small number of patients and the high interpatient variability at baseline. Therefore, these observations should be examined along with the forthcoming results of 2 ongoing phase 3 trials, Efficacy Study of GS010 for the Treatment of Vision Loss up to 6 Months From Onset in LHON Due to the *ND4* Mutation (RESCUE) and Efficacy Study of GS010 for Treatment of Vision Loss From 7 Months to 1 Year From Onset in LHON Due to the *ND4* Mutation (REVERSE), in which 76 patients with G11778A-*ND4* LHON were treated with rAAV2/2-*ND4* at a dose of 9×10^{10} vg per eye.

Conclusions

Ocular gene therapy with rAAV2/2-*ND4* in these 15 patients appeared to be overall safe and well tolerated with mostly mild, self-limited intraocular inflammation responsive to treatment that might be prevented using prophylactic immunosuppressive therapy in future trials. These results support the continuation of gene therapy in LHON and other families of eye diseases.

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Author Contributions: Dr Bouquet had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Bouquet, Galy, Fitoussi, Sahel, Thomasson.
Acquisition, analysis, or interpretation of data: Bouquet, Vignal Clermont, Galy, Fitoussi, Blouin, Munk, Valero, Meunier, Katz, Thomasson.
Drafting of the manuscript: Bouquet, Blouin.
Critical revision of the manuscript for important intellectual content: All authors.
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