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Reports

Safety of rAAV2/2-*ND4* Gene Therapy for Leber Hereditary Optic Neuropathy

Leber hereditary optic neuropathy (LHON) is the most commonly recognized mitochondrial disease. It typically occurs in young male adults, causing painless, acute, and profound vision loss. It presents asynchronously with the second eye almost always involved within weeks or months, a phenotypic declaration nearly pathognomonic for LHON. Visual prognosis is poor and therapy wanting.[1]

Leber hereditary optic neuropathy is caused by mutations in mitochondrial genes encoding proteins of the respiratory chain complex I. Approximately 70% of subjects with LHON carry the point mutation G11778A in the *ND4* gene encoding NADH dehydrogenase protein subunit 4 (*ND4*), accounting for the most severe phenotype.[2] Retinal ganglion cells are primarily affected by this mitochondrial dysfunction, leading to apoptotic cell death and ensuing optic nerve atrophy.[1]

Our therapy is based on a technology that demonstrably rescued an induced defect in respiratory chain complex I in rat retinas and prevented retinal ganglion cell degeneration.[3] rAAV2/2-*ND4* (GS010) is a recombinant, replication-defective, adeno-associated virus, serotype 2, containing a modified cDNA encoding the human wild-type mitochondrial ND4 protein and supporting its allotopic expression,[3] that is, the expression of a mitochondrial gene by the nucleus and subsequent co-translation and importation of the wild-type protein into the mitochondrial matrix. rAAV2/2-*ND4* was investigated in an open-label single-center Phase I/II clinical trial that included 4 dose-escalation cohorts (9×10^9 vector genomes [vg]/eye, 3×10^{10} vg/eye, 9×10^{10} vg/eye, 1.8×10^{11} vg/eye) and an extension cohort (9×10^{10} vg/eye). Fifteen subjects with LHON carrying the *ND4*-G11778A mutation were prospectively enrolled. Each subject received a single intravitreal injection of rAAV2/2-*ND4* in the worse-seeing eye. The study design included an initial follow-up period of 48 weeks, followed by longer-term follow-up for an additional 4 years. The primary

Table 1. Ocular Treatment-Emergent Adverse Events and Outcomes of the Most Common Treatment-Emergent Adverse Events

	rAAV2/2-*ND4* doses				
	9×10^9 *vg/eye* (*n* = 3)	3×10^{10} *vg/eye* (*n* = 3)	9×10^{10} *vg/eye* (*n* = 6)*	1.8×10^{11} *vg/eye* (*n* = 3)	All (N = 15)
All Ocular TEAEs	4/3	10/3	29/6	11/3	56/15
Anterior chamber inflammation	0	2/2	10/6	2/2	14/10†
Subconjunctival hemorrhage	0	0	3/3	0	3/3‡
Allergic conjunctivitis	1/1	0	0	0	1/1
Punctate serous detachment	0	0	0	1/1	1/1#
Eye pain	0	1/1	1/1	0	2/2#
Keratitis	0	1/1	4/4	2/2	7/7
Ocular hypertension	2/2	3/3	2/2	3/2	10/9§
Vitreous hemorrhage	0	0	2/2	0	2/2
Vitritis	1/1	3/2	6/5	3/3	13/11#
Positive Seidel test	0	0	1/1	0	1/1
Cataract extraction (elective)	1/1	0	1/1	0	2/2
Outcome of the 3 Most Common Ocular TEAEs					
Anterior chamber inflammation					
Recovered	0	2/2	10/6	2/2	14/10
Ongoing	0	0	0	0	0
Vitritis					
Recovered	0	3/2	6/5	3/3	12/10
Ongoing	1/1	0	0	0	1/1‖
Ocular hypertension					
Recovered	2/2	3/3	2/2	3/2	10/9
Ongoing	0	0	0	0	0

TEAE = treatment-emergent adverse event; VG = vector genomes.
Results are presented as N events/N subjects.
*Includes subjects of cohorts 3 and 5.
†Two events were considered unrelated to rAAV2/2-*ND4* or the procedure; the remaining 12 events were considered to be probably related to rAAV2/2-*ND4*.
‡All events were considered to be probably related to the procedure.
§Of 10 events, 6 were considered to be related to rAAV2/2-*ND4*, and 4 were related to the procedure.
‖Subject withdrew consent after week 48.
#All events were considered to be probably related to rAAV2/2-*ND4*.

objective was the safety and tolerability of escalating doses of rAAV2/2-*ND4*. Secondary objectives included bio-dissemination and immunogenicity of rAAV2/2-*ND4* and evaluation of visual functions. The study received approval of the French Ethics Committee and adhered to the tenets of the Declaration of Helsinki; it was registered on Clinicaltrial.gov (NCT02064569).

Subjects were mostly male (n = 13) with an average age of 47 years. Vision loss duration was heterogeneous, ranging from 8 to 271 months at enrollment (Table S1, available at www.aaojournal.org). The incidence, type, severity, and presumed causality of treatment-emergent adverse events (TEAEs) were collected for each subject at all study visits. Each event was recorded as a separate adverse event even when occurring simultaneously (e.g., anterior and vitreous inflammation). Visual parameters measured included best-corrected visual acuity (BCVA), Pelli-Robson contrast sensitivity, 15-hue color vision, Humphrey Visual Field 24-2, Octopus perimetry, microperimetry, visual evoked potentials, and pattern electroretinogram.

At week 96, 96 TEAEs were reported involving all 15 subjects, including 40 systemic TEAEs and 56 ocular TEAEs, consistent with previous studies using intravitreal injections (Table 1).[4,5] There were no unexpected TEAEs, no serious adverse events related to the treatment or procedure, and no suspected unexpected serious adverse reactions. No deaths and no TEAEs leading to study discontinuation were reported. Ninety of the 96 TEAEs (94%) were mild in intensity. Of the 56 ocular TEAEs, 2 events occurred in 1 untreated eye and 2 were elective cataract extraction. Thirty-four (61%) and 18 (32%) TEAEs were considered treatment and procedure related, respectively. Fifty-one ocular TEAEs (91%) were mild. One moderate event of intraocular pressure (IOP) elevation (34 mmHg) occurred in the only subject who did not receive a pretreatment IOP-lowering agent. Another subject concomitantly experienced an event of ocular pain and moderate elevation of IOP (38 mmHg), followed by 2 severe events: anterior chamber inflammation and vitritis.

The most frequent ocular TEAEs were intraocular inflammation and IOP elevation. Twenty-seven events of intraocular inflammation were reported in 13 subjects, starting from 7 to 541 days posttreatment. All were mild, except 2 severe events that occurred in a single subject. All were deemed probably related to treatment, except for 2 TEAEs that occurred in 1 untreated eye (subject had a history of idiopathic uveitis). Most affected subjects were treated for their ocular inflammation with topical anti-inflammatory agents. Two subjects were given oral steroids: the subject with severe intraocular inflammation and another subject with mild vitritis.

Ten events of IOP elevation following intravitreal injection were reported in 9 subjects, starting 4 hours to 30 days after injection. Most cases were mild, except for 2 subjects with moderate IOP elevation. Day-of-treatment IOP elevation was deemed to be procedure related (due to the injection). Delayed IOP elevation was deemed treatment related, and such subjects developed subsequent intraocular inflammation, precipitating steroid use.

All ocular events resolved spontaneously or after appropriate therapy with IOP-lowering or anti-inflammatory therapy, except for 1 subject with ongoing mild vitritis (0.5+ vitreous cell, no vitreous haze, no treatment required at last visit), subsequently lost to follow-up but without documented worsening of TEAE. At week 96, no related TEAEs required ongoing treatment. No anatomic sequelae were documented by fundus examination or spectral domain OCT, and no vision loss was reported due to treatment or TEAEs. A total of 40 systemic TEAEs were reported over 96 weeks; none were related to rAAV2/2-*ND4* or the study procedures (Table S2, available at www.aaojournal.org).

Although this study was neither designed nor powered to ascertain efficacy, a clinically significant improvement in BCVA was noted in the treated eyes of 6 of 14 subjects at week 96 (similar proportions at week 48 and week 78). A between-eye difference in visual acuity change from baseline favoring the treated eye was observed at week 96 in the subset of subjects with disease duration $\leq$2 years and BCVA $\geq$20/12000 at inclusion (−0.278 logarithm of the minimum angle of resolution [logMAR] [+14 Early Treatment Diabetic Retinopathy Study {ETDRS} letters] 95% confidence interval [CI], −0.853 to +0.297). This between-eye difference was also observed at week 48 and week 78 (−0.338 logMAR [+17 ETDRS letters] 95% CI, −0.856 to +0.180 and −0.398 logMAR [+20 ETDRS letters] 95% CI, −1.021 to +0.225, respectively).

Our study demonstrates that rAAV2/2-*ND4* is safe and well tolerated 2 years after a single unilateral intravitreal administration. Our 2 ongoing Phase III clinical studies in similar subjects, RESCUE (vision loss duration of $\leq$6 months) and REVERSE (vision loss duration of >6 months to 1 year), should help refine and inform the trends of improvement in visual outcome that we have reported and further validate the safety and tolerability of allotopic expression in mitochondrial disease.

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