ORIGINAL ARTICLE

Gene Therapy in Patients with the Crigler–Najjar Syndrome

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ABSTRACT

BACKGROUND

Patients with the Crigler–Najjar syndrome lack the enzyme uridine diphosphoglucuronate glucuronosyltransferase 1A1 (UGT1A1), the absence of which leads to severe unconjugated hyperbilirubinemia that can cause irreversible neurologic injury and death. Prolonged, daily phototherapy partially controls the jaundice, but the only definitive cure is liver transplantation.

METHODS

We report the results of the dose-escalation portion of a phase 1–2 study evaluating the safety and efficacy of a single intravenous infusion of an adeno-associated virus serotype 8 vector encoding UGT1A1 in patients with the Crigler–Najjar syndrome that was being treated with phototherapy. Five patients received a single infusion of the gene construct (GNT0003): two received 2×10^{12} vector genomes (vg) per kilogram of body weight, and three received 5×10^{12} vg per kilogram. The primary end points were measures of safety and efficacy; efficacy was defined as a serum bilirubin level of 300 μmol per liter or lower measured at 17 weeks, 1 week after discontinuation of phototherapy.

RESULTS

No serious adverse events were reported. The most common adverse events were headache and alterations in liver-enzyme levels. Alanine aminotransferase increased to levels above the upper limit of the normal range in four patients, a finding potentially related to an immune response against the infused vector; these patients were treated with a course of glucocorticoids. By week 16, serum bilirubin levels in patients who received the lower dose of GNT0003 exceeded 300 μmol per liter. The patients who received the higher dose had bilirubin levels below 300 μmol per liter in the absence of phototherapy at the end of follow-up (mean [±SD] baseline bilirubin level, 351±56 μmol per liter; mean level at the final follow-up visit [week 78 in two patients and week 80 in the other], 149±33 μmol per liter).

CONCLUSIONS

No serious adverse events were reported in patients treated with the gene-therapy vector GNT0003 in this small study. Patients who received the higher dose had a decrease in bilirubin levels and were not receiving phototherapy at least 78 weeks after vector administration. (Funded by Genethon and others; ClinicalTrials.gov number, NCT03466463.)

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This is the *New England Journal of Medicine* version of record, which includes all *Journal* editing and enhancements. The Author Accepted Manuscript, which is the author's version after external peer review and before publication in the *Journal*, is available at PubMed Central.

This article was updated on August 17, 2023, at NEJM.org.

N Engl J Med 2023;389:620-31.
DOI: 10.1056/NEJMoa2214084
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The Crigler–Najjar syndrome is a recessively inherited metabolic disorder of the liver caused by variants in *UGT1A1*, the gene encoding uridine diphosphoglucuronate glucuronosyltransferase 1A1, an enzyme that conjugates bilirubin.[1-4] Patients with severe disease have jaundice because of excess levels of accumulated unconjugated bilirubin, which, if the levels exceed 300 μmol per liter, may cause irreversible neurologic injury and death.[5] Prolonged daily phototherapy partially and transiently controls the jaundice, but the only definitive cure is liver transplantation.[6]

Therapeutic transplantation of allogeneic hepatocytes has been attempted, with limited and only short-term efficacy.[7,8] Liver-directed gene therapy with adeno-associated virus (AAV) vectors holds the potential for long-lasting correction of a variety of diseases.[9-17] We hypothesized that, on the basis of promising preclinical results,[18-20] serum bilirubin levels would be reduced by gene therapy with an AAV vector that includes *UGT1A1* in patients with the Crigler–Najjar syndrome. Here, we report the safety and efficacy data from the dose-escalation portion of a multicenter, open-label, phase 1–2 study of gene therapy with an AAV serotype 8 vector (GNT0003).

Methods

Study Design

We performed an open-label, multicenter, dose-escalation, phase 1–2, nonrandomized clinical study to evaluate the safety and efficacy of an intravenous injection of GNT0003 (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The study protocol (available at NEJM.org) was approved by the relevant national regulatory authorities and an institutional review board at each site. All the patients in the study provided written informed consent for enrollment into one of the two dose cohorts in which they would receive a single intravenous infusion of GNT0003: cohort 1 received 2×10^{12} vector genomes (vg) per kilogram of body weight, and cohort 2 received 5×10^{12} vg per kilogram.

We planned to enroll a maximum of six adult patients in the dose-escalation phase of the study (up to three in each cohort), with the option of stopping enrollment in each cohort if we observed a lack of efficacy in the first two patients and on the basis of advice from the data and safety monitoring board. Laboratory assessments were performed at a centralized biochemistry laboratory at baseline and during the 48 weeks after GNT0003 administration. At the end of the 48-week follow-up, patients were enrolled in a long-term follow-up study. Blood tests were also performed locally, at a laboratory at the enrollment site. Local laboratory data are available for the period 80 weeks (78 weeks for two of the patients) after GNT0003 infusion.

End Points

The primary end point was the safety of GNT0003, measured as the incidence of adverse events or serious adverse events, which were evaluated as changes in laboratory measures, vital signs, and findings on physical examination at baseline and after GNT0003 administration. The primary efficacy end point was defined as a serum level of total bilirubin of 300 μmol per liter or lower 7 days after interruption of daily phototherapy, which occurred at week 16 after administration of GNT0003. Secondary and exploratory end points included measures of the emergence of and trends in specific humoral and cellular immune responses against the infused vector or encoded transgene and vector shedding in body fluids.

Eligibility Criteria

We enrolled persons who were at least 18 years of age who had severe Crigler–Najjar syndrome and received daily phototherapy (≥6 hours per day) to maintain a total bilirubin level of 300 μmol per liter or lower. Patients were not eligible if they had received a liver transplant or had detectable serum neutralizing antibodies against AAV8 or advanced fibrosis detected through liver biopsy or transient elastography. Additional eligibility criteria are provided in the Supplementary Appendix.

Treatment and Monitoring Schedule

The patients received sirolimus (initial dose, 2 mg per day), with the dose adjusted to reach trough blood levels of 4 to 12 μg per liter, 1 week before vector administration through week 12 after administration. In addition, on the day before vector administration, patients received an intravenous dose of 100 mg of methylprednisolone and started a course of daily oral prednisone (40 mg), which was tapered beginning at week 3 and was

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suspended at week 8. Overnight phototherapy was maintained until week 14 and then tapered to cessation at week 16; the efficacy end point was evaluated at week 17 (Fig. S2 in the Supplementary Appendix). In the event of a suspected T-cell response against the vector, measured as an increase in alanine aminotransferase (ALT) or bilirubin levels, a supplementary course of prednisone, starting at a dose of 60 mg per day with tapering to discontinuation within 1 to 2 months,[21] could be initiated at the discretion of the investigator. Similarly, in the event of an increase in liver-enzyme levels with or without an increase in bilirubin levels occurring during the tapering of sirolimus treatment, immunosuppression could be prolonged at the discretion of the investigator.

All the patients underwent extensive biochemical testing at a centralized laboratory at weeks 1, 4, 8, 12, 16, 17, 24, 36, and 48 after treatment. They also underwent more frequent blood testing at a local laboratory up to 80 weeks after GNT0003 infusion to strictly monitor markers of efficacy and toxicity, in particular the levels of total bilirubin (normal value, <21 μmol per liter) and ALT (normal value, <45 IU per liter).

STATISTICAL ANALYSIS

This is a phase 1–2, dose-finding study. The data analysis in this type of study is descriptive in nature. Biochemical and hematologic test results were followed in a longitudinal manner at local and centralized laboratories, with blood sampling performed twice weekly until week 28 and thereafter with variable frequency (based on clinical judgment) until week 80. When appropriate, data are reported as means (±SD). Data analysis and the generation of graphs were performed with GraphPad Prism software for Windows, version 9.3.1 (GraphPad Software).

RESULTS

STUDY POPULATION

The first patient was enrolled on December 4, 2018, and the data-cutoff date for the analysis was December 21, 2022. Five women 21 to 30 years of age with a mean (±SD) total bilirubin level at baseline of 314±80 μmol per liter and an unconjugated bilirubin level of 280±65 μmol per liter who were receiving between 7 and 12 hours of overnight phototherapy to maintain steady bilirubin levels were enrolled in the escalation-phase study (Fig. S3). The degrees of previous exposure to phototherapy differed among the patients, and it was requested that they maintain the same type, time, and mode of phototherapy before and after GNT0003 administration, along with other treatments, such as phenobarbital (Table 1). The two patients who underwent liver biopsy during the baseline observation period were found to have mild fibrosis and inflammation and a vascular pattern consistent with obliterative portal venopathy (Table 1).

EFFICACY OF GNT0003

Low-Dose Cohort

After the infusion of GNT0003, Patient 1 had an initial decrease in total bilirubin level to a minimum level of 97 μmol per liter at week 2. The bilirubin levels rebounded to 272 μmol per liter by week 16, and the decision was made to not attempt to withdraw phototherapy. At week 21, her bilirubin level (357 μmol per liter) had returned to the pretreatment level, and it remained high during follow-up (Fig. 1). This patient received a reactive course of oral prednisone at week 8 and three intravenous boluses of 100 mg of methylprednisolone at week 14 in response to changes in ALT levels that occurred after the initial courses of treatment with prednisone and sirolimus, respectively, had been suspended in accordance with the protocol (Fig. 2).

Patient 2 had a slow decrease in bilirubin levels, reaching 121 μmol per liter at week 14. At week 16, the bilirubin level was 148 μmol per liter, which prompted the suspension of phototherapy. The bilirubin level had increased to 215 μmol per liter by week 17, and phototherapy was restarted. At week 80, the total bilirubin level was 340 μmol per liter (Fig. 1). This patient had a transient increase in ALT levels at week 20 (Fig. 2).

High-Dose Cohort

Patient 3 had a progressive decrease in bilirubin levels, to 64 μmol per liter at week 16. Phototherapy was suspended, and at week 17 the bilirubin level was 63 μmol per liter (Fig. 1). At week 12, at the time that treatment with sirolimus was suspended, the ALT level increased from 15 IU per liter to 47 IU per liter, and an extra course of prednisone was initiated. At week 25, the bilirubin levels began to increase, which led to the deci-

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Table 1. Baseline Characteristics of the Patients.*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Female	Female	Female	Female	Female
Age (yr)	23	26	26	18	27
DNA mutation	NM_000463.3:c.[1156G→T];[1156G→T]	NM_000463.3:c.[1220del];[1220del]	NM_000463.3:c.[1006C→T];[1130G→T]	NM_000463.3:c.[1184G→T];[1184G→T]	NM_000463.3:c.[1006C→T];[1304+1G→T]
Amino acid substitution	NP_000454.1:p.[Val386Phe];[Val386Phe]	NP_000454.1:p.[Lys407Argfs*5];[Lys407Argfs*5]	NP_000454.1:p.[Arg336Trp];[Gly377Val]	NP_000454.1:p.[Gly395Val];[Gly395Val]	NP_000454.1:p.[Arg336Trp];[splicing site]
Phototherapy device	Philips Rebel LXML-PR01-0500	BiliLed Blue Night 200	Cree Royal Blue LED	Philips TL/52 and Cree Royal Blue LED	Philips TL/52 and Cree Royal Blue LED
Type of blue-light emission	LED	LED	LED	Neon and LED	Neon and LED
Wavelength of irradiance (nm)	445–450	460–475	455	450 (neon), 455 (LED)	450 (neon), 455 (LED)
Number of bulbs	12 pads with 30 LED bulbs each	13 pads with 28 LED bulbs each	23 tubes	6 tubes (neon), 12 tubes (LED)	22 tubes (neon), 10 tubes (LED)
Skin exposure (%)	45	45	45	45	45
Distance from light source (cm)	70	80	40	40	60
Mean daily exposure (hr)	12.0	8.9	7.6	7.9	7.6
Irradiance at skin ($\mu W/cm^2$/nm)	150	120	NA	NA	NA
Phenobarbital treatment (mg/day)	—	—	100	—	100
Total bilirubin level (μmol/liter)	355±32	235±16	322±37	393±40	268±50
Unconjugated bilirubin level (μmol/liter)	341±37	218±18	289±34	371±40	264±54
ALT level (IU/liter)	49±11	37±13	110±71	68±16	44±39
AST level (IU/liter)	46±5	21±4	50±21	37±7	32±19
GGT level (IU/liter)	104±8	39±8	62±30	35±5	94±22
Relevant medical history	—	—	Gallstones, cholecystectomy	—	—
Findings on transient elastography (kPa)†	4.8	3.4	4.9	5.7	4.2
Liver-biopsy findings	NP	NP	Mild inflammation, mild fibrosis, dilatation of the lumen of the sinusoids and of some centrilobular veins	NP	No inflammation, mild fibrosis, dilatation of the lumen of the sinusoids and of some centrilobular veins

* Plus–minus values are means ±SD. ALT denotes alanine aminotransferase, AST aspartate aminotransferase, GGT γ-glutamyltransferase, LED light-emitting diode, NA not available, and NP not performed.
† Transient elastography measures the stiffness of the liver.

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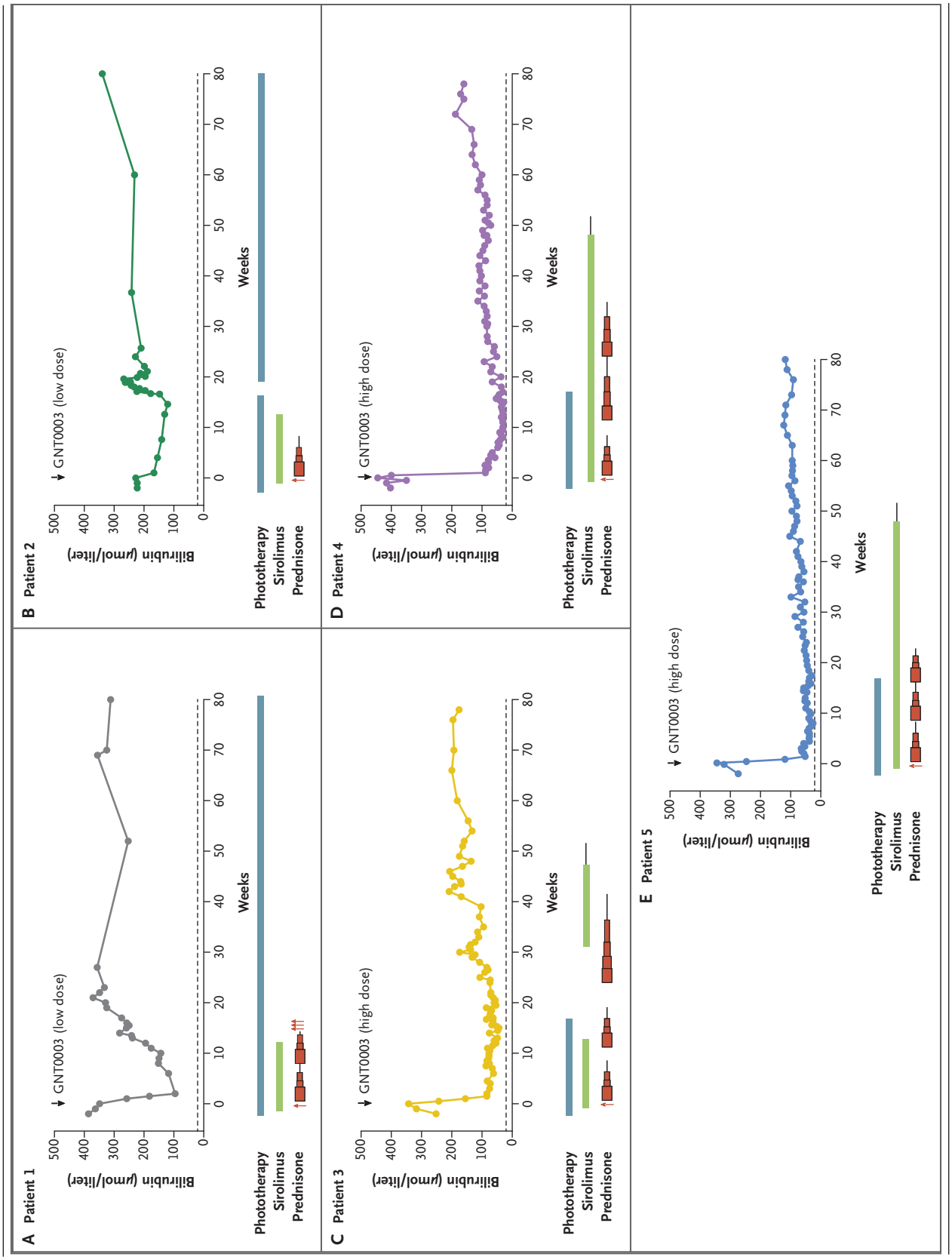

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Figure 1 (facing page). Levels of Total Serum Bilirubin in Both Dose Groups.

Patients 1 and 2 received the lower dose of GNT0003 (2×10^{12} vector genomes [vg] per kilogram of body weight), Patients 3, 4, and 5 received the higher dose (5×10^{12} vg per kilogram). The blue box below each graph represents the use of phototherapy over time, and the green and red boxes indicate the duration (length) and dose (box size) of sirolimus (green) and prednisone (red) over time. Red arrows indicate intravenous boluses of 100 mg of methylprednisolone. The dashed line indicates the upper limit of the normal range. In Patient 1, the total bilirubin level measured at week 16 did not allow for discontinuation of phototherapy. Patients 3, 4, and 5 discontinued phototherapy and were still not receiving it at the end of follow-up.

sion to administer an additional course of glucocorticoids; in addition, at week 30, treatment with sirolimus was resumed and was maintained until week 48, then tapered to discontinuation at week 52 (Fig. 2). At week 78 (the final follow-up visit for this patient), the bilirubin level was 176 μmol per liter in the continued absence of phototherapy.

Patient 4 had a progressive decrease in bilirubin levels from baseline, to 48 μmol per liter at week 16. Phototherapy was suspended at week 16, and at week 17 the bilirubin level was 31 μmol per liter (Fig. 1). At week 11, an increase in the ALT level from 20 to 44 IU per liter prompted the administration of a reactive course of prednisone. On the basis of the experience with Patients 1 and 3, it was decided to continue treatment with sirolimus until week 48 and then taper to discontinuation at week 52 (Fig. 2). At week 78 (the final follow-up visit for this patient), the bilirubin level was 160 μmol per liter in the absence of phototherapy.

Patient 5 had a progressive decrease in bilirubin levels, to 38 μmol per liter at week 16. Phototherapy was suspended at week 16, and at week 17 the bilirubin level was 38 μmol per liter (Fig. 1). At week 8, the ALT level increased from 7 IU per liter to 35 IU per liter, and at week 15, the ALT level increased from 16 IU per liter to 32 IU per liter; each of these increases led to the initiation of an extra course of prednisone. Treatment with sirolimus was continued until week 48 and then tapered to discontinuation at week 52 (Fig. 2). At week 80, the bilirubin level was 117 μmol per liter in the absence of phototherapy.

Among the patients in the high-dose cohort, the mean bilirubin level was 351±56 μmol per liter at baseline. At the end of the follow-up, the mean bilirubin level was 149±33 μmol per liter in the absence of phototherapy.

SAFETY

None of the patients had serious adverse events. No hypersensitivity reactions occurred after the infusion of GNT0003. The mean total bilirubin level had decreased to 114±98 μmol per liter at 16 weeks among the patients treated with the highest dose (Fig. S4A). The ALT level decreased from a mean baseline value of 58±14 IU per liter to 17±4 IU per liter at week 4 and stabilized within the normal range in all patients who had a response to treatment (Fig. S4B).

Four patients had increases in ALT levels at different points during follow-up, which were interpreted as potential T-cell–mediated reactions against the vector.[22] Other nonserious adverse events are reported in Table 2. Patient 1 became pregnant at 24 weeks and 45 months after gene therapy (details are provided in the Supplementary Appendix).

The GNT0003 vector genomes were detectable in plasma and urine within 72 hours after vector infusion. In all patients, clearance of vector occurred in a dose-dependent manner in all tested samples, with plasma showing the slowest clearance (Fig. S5).

IMMUNE RESPONSES TO VECTOR AND TRANSGENE

Humoral immune responses to the AAV8 capsid were similar in all five patients. Levels of total IgG against AAV8 peaked between week 12 and week 24 (Fig. 3A). Levels of anti-AAV8 IgM peaked at week 4 before returning to baseline (Fig. 3B). In all the patients, persistent high titers of anti-AAV8 neutralizing antibodies developed (Fig. 3C). Antibodies directed against the UGT1A1 transgene product were not consistently detected (Tables S1 and S2). T-cell–mediated immune responses against UGT1A1 and AAV8 were mostly undetectable (Tables S3 and S4), probably because of the concomitant administration of immunosuppressive drugs.

DISCUSSION

Few liver-based diseases have been targeted by gene therapy in clinical studies, and attempts at

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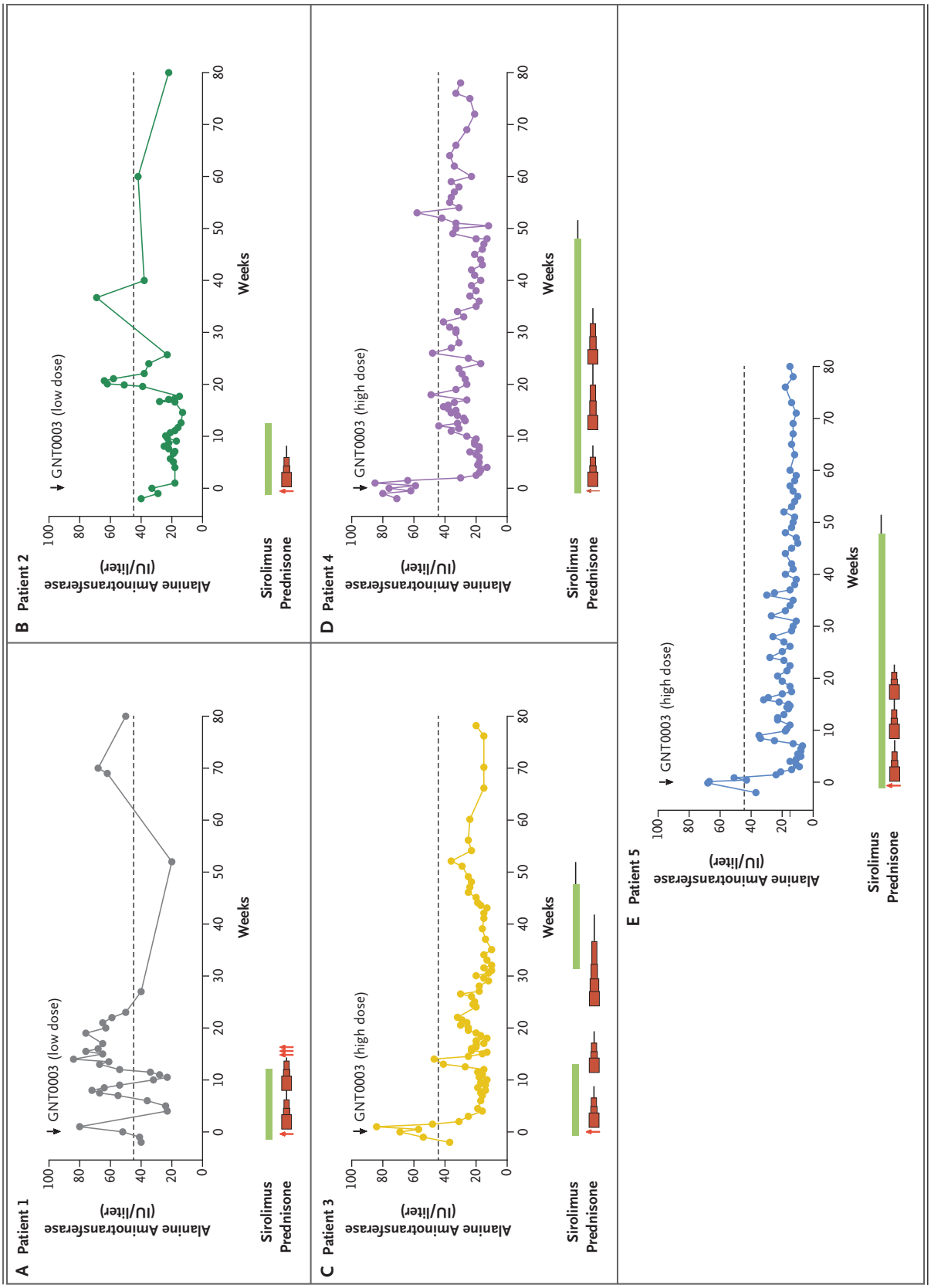

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Figure 2 (facing page). Levels of Alanine Aminotransferase in Both Dose Groups.

The colored boxes at the bottom of the graphs indicate the duration (length) and the dose (box size) of sirolimus (green) and prednisone (red) over time. Red arrows indicate intravenous boluses of 100 mg of methylprednisolone. The dashed line indicates the upper limit of the normal range.

treatment have met with variable success.[23] Gene therapy has been effective in the treatment of hemophilia, in which the rate of bleeding episodes is durably decreased after receipt of AAV-mediated gene transfer.[12,24,25] The Crigler–Najjar syndrome is a candidate disease for gene-replacement therapies because it is a well-characterized monogenic disease and has a uniquely hepatic origin and because end points of efficacy are easy to measure.[26,27]

Our findings provide preliminary evidence that liver-directed gene transfer with GNT0003 is not associated with serious adverse events and can correct bilirubin levels, allowing for discontinuation of phototherapy. More generally, our findings support the possibility of long-term correction of a genetic disease caused by inactive variants in a gene that encodes a nonsecreted liver protein (UGT1A1 is a membrane-bound protein residing in the endoplasmic reticulum of hepatocytes).

Previous clinical reports of hepatocyte transplantation and auxiliary liver transplantation indicate that restoring 5 to 10% of UGT1A1 activity should be sufficient to decrease bilirubin levels by 30 to 40% while the patient is receiving phototherapy.[7,8,28] In our study, GNT0003 at a dose of 5×10^{12} vg per kilogram reduced bilirubin levels to as low as 20 to 30% of baseline values after suspension of phototherapy. This finding suggests that the percentage of transduced hepatocytes in these patients was at least 5 to 10%, which is consistent with findings from studies in nonhuman primates injected with similar doses of AAV8 vectors.[29] Alternatively, perhaps the percentage was lower, with some hepatocytes expressing supraphysiologic levels of UGT1A1, as has been observed in animal models of gene transfer.[18] A vector dose of 2×10^{12} vg per kilogram elicited only a transient reduction in bilirubin levels, a finding consistent with data obtained in

Table 2. Adverse Events during Treatment.*

Event	Low Dose (N=2)	High Dose (N=3)	Overall (N=5)
	number of events		
Any adverse event during treatment	25	17	42
Blood and lymphatic system disorders	1	1	2
Anemia	1	0	1
Increased tendency to bruise	0	1	1
Gastrointestinal disorders	0	1	1
Upper abdominal pain	0	1	1
General disorders and administration-site conditions	3	0	3
Asthenia	2	0	2
Influenza-like illness	1	0	1
Infections and infestations	5	3	8
Covid-19	2	1	3
Cytomegalovirus infection	1	0	1
Nasopharyngitis	1	0	1
Pharyngitis	1	0	1
Rhinitis	0	1	1
Soft-tissue infection	0	1	1
Laboratory abnormalities	11	8	19
Increase in alanine aminotransferase level†	5	4	9
Increase in aspartate aminotransferase level†	5	4	9
Increase in white-cell count	1	0	1
Metabolism and nutrition disorders	2	0	2
Hypokalemia	1	0	1
Increase in appetite	1	0	1
Musculoskeletal and connective-tissue disorders	0	2	2
Pain in limbs	0	1	1
Rhabdomyolysis	0	1	1
Nervous system disorders	2	2	4
Headache	2	2	4
Skin and subcutaneous-tissue disorders	1	0	1
Rash	1	0	1

* The lower dose of GNT0003 was 2×10^{12} vector genomes [vg] per kilogram of body weight, and the higher dose was 5×10^{12} vg per kilogram. Covid-19 denotes coronavirus disease 2019. Terms used in the table are adapted from the system organ classes and preferred terms in the *Medical Dictionary for Regulatory Activities*, version 23.1.

† Only values higher than the upper limit of the normal range were considered.

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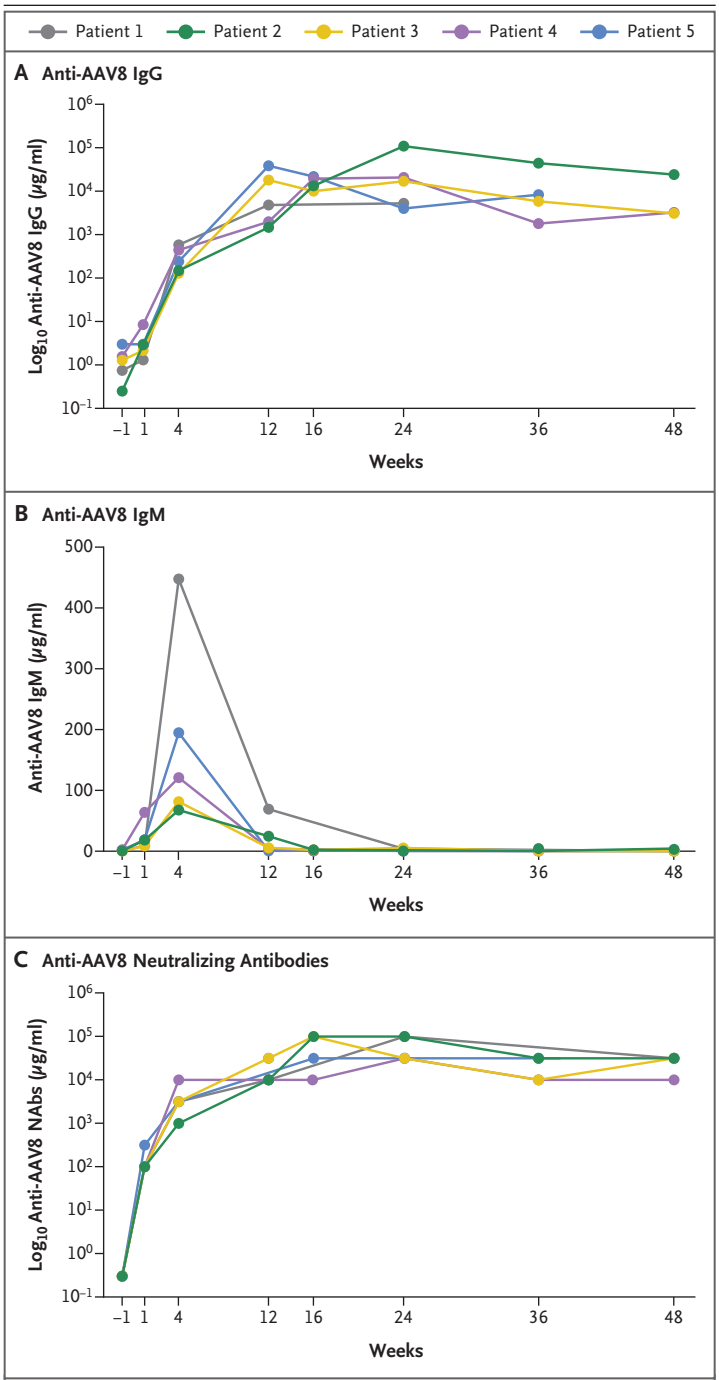

Figure 3. Humoral Response to AAV8 Vector Capsid after the Infusion.
The dots represent the single values. AAV8 denotes adeno-associated virus serotype 8, and NAbs neutralizing antibodies.

animal models that have received subtherapeutic doses.[18,19]

Despite presenting with nearly normal liver architecture, most patients with the Crigler–Najjar syndrome have elevated liver-enzyme levels at baseline (as was true of all the patients in this study).[30] This did not appear to affect the safety or the efficacy of GNT0003: after vector administration, and concomitant with the decrease in bilirubin levels, liver-enzyme levels returned to normal. Our results suggest that GNT0003 restored UGT1A1 expression in hepatocytes and that it had a beneficial effect on the mild, ongoing liver disease that is typical of patients with the Crigler–Najjar syndrome.[30]

AAV vectors can trigger immune responses in humans[31]; these responses are usually managed with immunosuppression.[12,14-17,32] Having observed an increase in ALT levels in Patients 1 and 3 at week 12, we amended the protocol to extend the sirolimus regimen to 52 weeks after GNT0003 administration on the basis of the recurrence of elevations in liver-enzyme levels at the time of interruption of the initial 12-week course of the drug. Late recurrence of immune responses to AAV, leading to prolonged immunomodulation, has been observed in other studies.[12,33]

We recorded no drug-related serious adverse events at the time of the infusion or during follow-up, perhaps because the highest vector dose was lower than those previously associated with severe reactions. Most of the patients had a mild elevation in ALT levels, which responded to a reactive course of glucocorticoid treatment.[22] After the GNT0003 infusion, ALT levels decreased to within the normal range in all the patients who had a response to the treatment. It is possible that this initial decrease in ALT levels lowered the threshold for the decision to initiate reactive glucocorticoid treatment in the case of a suspected T-cell response against the vector, a decision that was made at the discretion of the investigator. We did not detect cellular immune responses against the AAV capsid as described in previous studies.[13-15,17,25] Patients 4 and 5 had low levels of bilirubin until the end of the follow-up, despite some mild late loss of efficacy, possibly

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related to a delayed immune response after discontinuation of sirolimus treatment at week 52.

Sirolimus is an attractive immunosuppressant in the context of gene transfer in the liver because of its additional effect on the induction of regulatory T cells, which are essential to the tolerance of the transgene after its transfer into the hepatocyte through AAV vectors.[34-39] In addition, the positive effect of sirolimus on autophagy results in enhanced liver transduction.[40] We cannot exclude the possibility that sirolimus and other treatments received by the patients over the course of this study affected bilirubin levels; however, this seems unlikely, because bilirubin levels at the end of follow-up, after a minimum washout period of 6 months, remained unchanged.

Administration of a transient course of sirolimus, along with prophylactic and reactive use of glucocorticoids, was followed by long-term stabilization of transgene expression, although treatment with sirolimus did not block anti-AAV humoral immune responses. Because the Crigler–Najjar syndrome is a disease that arises in childhood, future studies should determine the persistence of long-term transgene expression in the developing liver and should test strategies that would allow repeat administration of AAV vectors.[29,41]

In gene transfer with the use of AAV vectors, a series of events needs to occur in order to achieve long-term expression of the transgene in the target cells. After the vector genome is stabilized in its episomal form,[42] expression is expected to be durable.[12,16] In some studies, a peak in expression has been observed shortly after vector administration, reaching a lower plateau thereafter.[12,35,43] It is hypothesized that this phenomenon is the result of the expression of transient forms of the AAV vector genome. Alternatively, epigenetic silencing may occur, or overexpression of the transgene may trigger endoplasmic reticulum stress[44] and transduced cell death.

The phototherapy regimen for the Crigler–Najjar syndrome is usually established empirically through observation of the response to treatment early in life[5]; the severity of the disease varies considerably and is influenced by genotype and by levels of serum bilirubin.[45,46] It is important that serum bilirubin levels do not exceed 350 to 400 μmol per liter[45]; spikes in levels of unconjugated bilirubin in the serum affect quality of life and can cause neurologic damage and death.[5,47] Because of this danger, our study protocol involved maintenance of the treatment that the patients had been receiving at the time of enrollment.

A limitation of our study is that disease severity was determined on the basis of bilirubin levels; measurements of residual enzymatic activity were not available. Furthermore, a more detailed evaluation of the daily dose of phototherapy (including hours of exposure, distance from the lamps, type and power of the bulbs, and extent of irradiance) and results of phenobarbital testing would have contributed to the overall evaluation of the severity of the condition in the patients.

Although our study is small, among the patients who received the dose of 5×10^{12} vg per kilogram, GNT0003 restored UGT1A1 activity to levels that permitted suspension of phototherapy, and the efficacy persisted at 18 months after the treatment. A test of replication in a larger, well-characterized cohort of patients will be important.

Supported by Genethon, the European Union Horizon 2020 plan (grant agreement 755225), and Telethon Foundation ETS.

Disclosure forms as provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

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