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ORIGINAL ARTICLE

Liver-Directed Adeno-Associated Virus–Mediated Gene Therapy for Mucopolysaccharidosis Type VI

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Abstract

BACKGROUND Mucopolysaccharidosis type VI (MPS VI) is an inherited multisystem lysosomal disorder due to arylsulfatase B (ARSB) deficiency that leads to widespread accumulation of glycosaminoglycans (GAG), which are excreted in increased amounts in urine. MPS VI is characterized by progressive dysostosis multiplex, connective tissue and cardiac involvement, and hepatosplenomegaly. Enzyme replacement therapy (ERT) is available but requires life-long and costly intravenous infusions; moreover, it has limited efficacy on diseased skeleton and cardiac valves, compromised pulmonary function, and corneal opacities.

METHODS We enrolled nine patients with MPS VI 4 years of age or older in a phase 1/2 open-label gene therapy study. After ERT was interrupted, patients each received a single intravenous infusion of an adeno-associated viral vector serotype 8 expressing ARSB. Participants were sequentially enrolled in one of three dose cohorts: low (three patients), intermediate (two patients), or high (four patients). The primary outcome was safety; biochemical and clinical end points were secondary outcomes.

RESULTS The infusions occurred without severe adverse events attributable to the vector, meeting the prespecified end point. Participants in the low and intermediate dose cohorts displayed stable serum ARSB of approximately 20% of the mean healthy value but returned to ERT by 14 months after gene therapy because of increased urinary GAG. Participants in the high-dose cohort had sustained serum ARSB of 30% to 100% of the mean healthy value and a modest urinary GAG increase that did not reach a concentration at

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which ERT reintroduction was needed. In the high-dose group, there was no clinical deterioration for up to 2 years after gene therapy.

CONCLUSIONS Liver-directed gene therapy for participants with MPS VI did not have a dose-limiting side-effect and adverse event profile; high-dose treatment resulted in ARSB expression over at least 24 months with preliminary evidence of disease stabilization. (Funded by the Telethon Foundation ETS, the European Commission Seventh Framework Programme, and the Isaac Foundation; ClinicalTrials.gov number, NCT03173521; EudraCT number, 2016-002328-10.)

Introduction

Maroteaux–Lamy syndrome, or mucopolysaccharidosis type VI (MPS VI), is an autosomal recessive lysosomal storage disease due to arylsulfatase B (ARSB) deficiency that results in multisystem accumulation of glycosaminoglycans (GAG).[1] Age of onset, disease manifestations, and rate of progression vary widely among patients with MPS VI. These patients develop hepatosplenomegaly due to GAG storage, short stature, severe skeletal and joint abnormalities, thickening of heart valves, and corneal clouding but there is no primary cognitive impairment.[2] Enzyme replacement therapy (ERT) consisting of weekly intravenous infusions of recombinant human ARSB (galsulfase) is available for MPS VI treatment. Although ERT reduces excretion of urinary GAG and hepatomegaly and improves endurance, it is less effective on other MPS VI complications — namely, cardiac valve disease, skeletal abnormalities, and corneal clouding.[3-9] ERT requires life-long parenteral infusions of protein at high costs and considerable inconvenience for patients and families. For MPS VI, hematopoietic stem cell transplantation (HSCT) has resulted in therapeutic outcomes similar to ERT. However, HSCT is hampered by graft failure, limited availability of human leukocyte antigen–matched donors, and significant risks related to preconditioning regimens and graft-versus-host disease.[10,11] These factors formed clinical equipoise for more definitive studies of treatments for MPS VI, including gene therapy.[12]

Liver-directed gene therapy based on adeno-associated viral (AAV) vectors is being investigated for disorders that require sustained delivery of therapeutic proteins from circulating plasma.[13] A single intravenous administration of an AAV8 encoding ARSB resulted in multiyear ARSB secretion,[14] reduced tissue and urinary concentrations of GAG, and improved motor activity and bone growth in small and large animal models of MPS VI.[15-20] Based on this premise, we conducted a phase 1/2 clinical study to investigate the safety and efficacy of AAV8-mediated, liver-directed *ARSB* gene transfer in patients with MPS VI.

Methods

STUDY DESIGN

This is a three-center, open-label, dose-escalation phase 1/2 study to investigate the safety and efficacy of AAV2/8.TBG.h*ARSB* in patients with MPS VI who are at least 4 years of age (ClinicalTrials.gov number, NCT03173521; EudraCT number, 2016-002328-10). The eligibility criteria are described in the Supplementary Methods in the Supplementary Appendix. The study design is available in the study protocol with the full text of this article at evidence.nejm.org.

Nine patients were enrolled into three cohorts, which sequentially received ascending doses of vector. The vector was administered in volumes of 30 to 210 ml over 120 to 240 minutes on a single occasion into a peripheral vein, according to the study protocol. The low dose (LD) cohort (three participants) received 6×10^{11} genome copies/kg, the intermediate dose (ID) cohort (two participants) 2×10^{12} genome copies/kg, and the high dose (HD) cohort 6×10^{12} genome copies/kg (four participants). For details, see the study protocol.

The study was approved by regulatory authorities (the Agenzia Italiana del Farmaco for the primary center) and by the institutional review boards of each site. One patient who was 18 years of age provided written informed consent. For the remaining younger patients, written informed consent was provided by their parents, and the patients provided assent.

This study was sponsored by the Telethon Foundation ETS and was designed by the first and last authors; data were collected by the site investigators (listed as authors) who vouch for the fidelity of the trial to the study protocol. Data were analyzed by the clinical and

statistical investigators and employees of the sponsor (all of whom are listed as authors of this article). The first two authors and the last author wrote the manuscript with critical input from all coauthors. The authors assume responsibility for the completeness and integrity of data and analyses. An independent drug safety monitoring board (DSMB) monitored safety and approved dose escalation.

VECTOR DESIGN AND PRODUCTION

The AAV2/8.TBG.h*ARSB* vector is a recombinant AAV serotype 8 vector containing a single-stranded AAV2 genome (AAV2/8) encoding the human ARSB (h*ARSB*) under the control of the thyroxine-binding globulin (TBG) promoter. The vector was produced according to good manufacturing practices.[20,21] Details about vector manufacturing are provided in the Supplementary Methods.

OUTCOMES

The primary objective of this study was to investigate the safety of the AAV2/8.TBG.h*ARSB* vector. Safety assessment included physical examination and vital signs, recording of adverse events, and laboratory testing, including immunological assays (for details, see the study protocol). Vector shedding was evaluated in serum, urine, stool, saliva, and semen (the latter was performed only for the single adult participant 01-001). The secondary objective was to investigate the efficacy of the AAV2/8.TBG.h*ARSB* vector by biochemical assays (urinary GAG concentrations and serum ARSB),[18,22] endurance tests, pulmonary and cardiac function tests, and the (Childhood) Health Assessment Questionnaire [(C)HAQ-DI] to assess quality of life.

Details on dose-limiting toxicity, grades of toxicity definitions, and safety and efficacy end points are included in the study protocol.

STATISTICAL ANALYSIS

Descriptions were made with mean (±SD), mean (±SE), or median (minimum to maximum or interquartile range) and count or percentages, as appropriate. No formal sample size calculation was done due to the nature of phase 1/2 clinical study. Because we opted for the standard 3 + 3 design and we considered three dose levels, the potential sample size ranges between 3 and 18 participants, depending on the observed toxicities. Given the descriptive nature of the study, no P-values are reported.

Results

STUDY PARTICIPANTS

Nine patients with MPS VI (five male and four female; Table 1) were enrolled into one of three dose cohorts. At treatment, patients ranged between 5 and 29 years (14±7, mean age ±SD) and they were diagnosed with MPS VI between 1 and 25 years of age by measuring enzyme activity and *ARSB* sequencing. All participants received ERT as 1 mg of galsulfase/kg per week for variable durations but at least 12 months before vector infusion. ERT was discontinued 7 to 14 days before gene therapy. Baseline serum ARSB was low or undetectable in all participants, with participant 01-008 showing higher residual serum ARSB. The median follow-up period after gene therapy was 36 months (minimum to maximum, 16 to 49), with an overall exposure to gene therapy of 300 months (Table 1). Herein, we present data of an interim analysis of the study at a time point that was selected arbitrarily.

SAFETY OUTCOMES

After vector infusion, AAV vector shedding was detected for up to 53 weeks, albeit at levels below the limit of quantification after a median of 13.8 weeks (interquartile range [IQR], 13.1 to 23.8) in serum, 4.5 weeks (IQR, 3.0 to 6.8) in saliva, 1.3 weeks (IQR, 0.6 to 23.0) in urine, and 13.0 weeks (IQR, 11.2 to 13.3) in stool in the HD cohort. Data on AAV vector shedding for the LD and ID groups are shown in Table S1. Vector shedding in semen in the adult participant (participant 01-001) was undetectable by 2 weeks after gene therapy (Fig. S1). Vector clearance from urine was faster than from any other body fluids. Persistence of vector DNA in sera and stool was dose dependent (Fig. S1).

No dose-limiting toxicity was observed and none of the participants developed cancer, autoimmune disease, or liver disease during the study. No acute cardiovascular adverse events and meaningful changes in vital signs were observed throughout the infusions. Six serious adverse events (all grade 3) and 60 adverse events (all grade $\leq$2) related to disease were reported (Table 2). Participant 01-004 developed fatigue and pain that were attributed to worsening of cervical spine stenosis because these symptoms resolved after surgical decompression. Participant 01-006 experienced worsening of

Table 1. Demographics and Main Clinical Features of Participants with MPS VI.*

Parameter	Low Dose			Intermediate Dose		High Dose			
	01-001	01-002	01-003	01-004	01-005	01-006	01-007	01-008	01-009
Sex	Male	Male	Male	Female	Male	Female	Male	Female	Female
ARSB genotype	c.236G>A/	c.943C>T/	c.962T>C/	c.962T>C/	c.304C>G/	c.589C>T/	c.236G>T/	c.1195T>C/	c.710C>T/
	c.236G>A	c.899-1142del	c. 962T>C	c. 962T>C	del exon7-8	c. 944G>A	c.1438G>T	c.1449A>T	c.114-1G>C
Amino acid change	p.Gly79Glu/	p.Arg315Ter	p.Leu321Pro/	p.Leu321Pro/	p.Arg102Gly	p.Arg197Thr/	p.Gly79Val/	p.Phe399Leu/	p.Ala237Val
	p.Gly79Glu	Exon deletion	p.Leu321Pro	p.Leu321Pro	Exon deletion	p.Arg315Gln	p.Asp480Tyr	p.Glu483Asp	Splicing mutation
Age, yr									
At diagnosis	25	1	1	6	1	3	2	2	2
At ERT start	25	4	3	6	2	3	2	3	2
At GT	29	16	13	16	14	10	10	10	5
Weight at GT, kg	53	37.5	23	25	32	24	25	26	20
Mean serum active ARSB at baseline, pg/ml†	9±19	Undetectable	41±41	21±20	13±21	37±53	4±12	332±64	15±28
Follow-up time after GT, mo	49	45	42	40	36	28	24	20	16

* Patients were all Caucasian (see the Supplementary Results). Data is reported by participant number per dose group. ARSB denotes arylsulfatase B, ERT enzyme replacement therapy, GT gene therapy, and MPS VI mucopolysaccharidosis type VI.

† Mean (±SD) values measured at day −1. For control individuals, the mean (±SD) normal range is 2119±632 pg/ml. *ARSB* genotype refers to NM_000046 for nucleotide sequence and NP_000037.2 for protein sequence.

glaucoma that was refractory to topical medications and resolved after bilateral trabeculectomy. Both events were deemed to be unrelated to the vector and considered to be related to disease progression, although consequences of ERT discontinuation cannot be formally excluded. Kidney function by monitoring of serum creatinine, albumin, total protein, and blood urea nitrogen was unaffected. Two patients showed proteinuria (urinary protein >1 g/24 hours) with normal kidney function and serum C3 and C4 complement concentrations that was attributable to the underlying disease rather than the vector. Increased serum thyroid-stimulating hormone with normal FT4 concentrations was observed in one participant (Table 2).

In participants 01-001 (LD cohort), 01-005 (ID cohort), and 01-006 and 01-008 (HD cohort), 1.5-fold higher than baseline serum alanine transaminase (ALT) was detected, but serum ALT remained below the upper normal limit of the healthy reference range (Fig. S2). Participants 01-001 and 01-005 started prednisolone after this increase was detected. In participant 01-001, the increased serum ALT did not return to pretreatment values after administration of prednisolone; values remained above the 1.5-fold increase over baseline but within the reference range even after prednisolone was discontinued at week 14 after gene therapy. The two HD participants restarted full-dose prednisolone after detection of increased serum ALT, which occurred either during tapering of prophylactic prednisolone in participant 01-006 or after its discontinuation for participant 01-008 (Fig. S2). Although no increased serum ALT was detected, greater T-cell reactivity to the AAV8 capsid was observed in participants 01-002 and 01-003 starting from weeks 8 and 6, respectively, and persisted up to the last time point of observation (week 19 for participant 01-002 and week 12 for participant 01-003). Conversely, no cell-mediated immune responses to AAV8 were observed in all remaining participants, although prednisolone might have masked an ongoing immune response (Table S2). AAV infusions at all doses were not associated with relevant changes in serum cytokine or chemokine concentrations (Fig. S3). Increased anti-ARSB T-cell reactivity was observed in participant 01-002 starting from week 8, which decreased at the last time point of observation (week 19), and in participant 01-005 at week 3 (Table S3). No correlation with circulating ARSB was observed in both participants. At baseline, total anti-ARSB antibodies were clearly measurable in participants 01-001 to 01-004, whereas they were below the limit of quantification in participant 01-006 and below the limit of detection in all other participants (Table S4).

Table 2. Summary of Serious Adverse Events and Adverse Events.*

Type	Grade	Total Events	No. of Patients			Participant No.
			LD	ID	HD	
Serious adverse event						
Glaucoma†,‡	3	2	0	0	2	01-006
Fatigue§	3	1	0	1	0	01-004
Heart failure¶	3	1	1	0	0	01-003
Pneumonia	3	2	2	0	0	01-002, 01-003
Adverse event						
Upper respiratory tract infection	2	9	7	0	2	01-001, 01-002, 01-003, 01-006, 01-009
Increased intraocular pressure	2	7	1	0	6	01-002, 01-006
Fatigue	2	3	3	0	0	01-001, 01-002, 01-003
Metabolic acidosis‖	2	2	0	0	2	01-006
Otitis media with effusion	2	2	1	1	0	01-001, 01-005
Anemia	2	1	0	0	1	01-009
Hidradenitis suppurativa	2	1	1	0	0	01-002
Increased serum bilirubin†,††	2	1	0	1	0	01-005
Inguinal hernia	2	1	1	0	0	01-002
Lower respiratory tract infection	2	1	0	1	0	01-005
Pharyngitis	2	1	1	0	0	01-001
Covid-19	2	2	1	0	1	01-001, 01-006
Headache	1	4	1	0	3	01-004, 01-006
Arthralgia	1	3	1	2	0	01-001, 01-004
Diarrhea	1	3	1	2	0	01-002, 01-004, 01-005
Increased protein/creatinine ratio	1	3	2	1	0	01-002, 01-003, 01-004
Abdominal pain	1	2	2	0	0	01-002, 01-003
Dyspnea	1	2	2	0	0	01-001, 01-003
Fecal and urinary incontinence	1	2	0	2	0	01-005
Sinus tachycardia	1	2	1	1	0	01-003, 01-005
Abdominal distension	1	1	1	0	0	01-001
Anxiety†	1	1	1	0	0	01-001
Dental caries	1	1	1	0	0	01-001
Increased serum TSH**	1	1	0	1	0	01-005
Neutropenia	1	1	0	0	1	01-009
Paresthesia	1	1	0	0	1	01-009
Sleep apnea†	1	1	1	0	0	01-001
Toothache	1	1	1	0	0	01-001

* The severity of adverse events was determined according to Common Terminology Criteria for Adverse Events version 5. None of the events were deemed by the investigators related to the study drug. HD denotes high dose, ID intermediate dose, LD low dose, Covid-19 coronavirus disease 2019, and TSH thyroid-stimulating hormone.

† Preexisting condition.

‡ Adverse events likely due to progression of the underlying condition.

§ Serious adverse event due to worsening of preexisting cervical spine stenosis.

¶ Worsening of a preexisting mitral valve disease that was corrected by surgery.

‖ Due to treatment with acetazolamide for glaucoma.

** Normal serum FT4 concentrations.

†† Mild unconjugated hyperbilirubinemia.

SERUM ARSB AND URINARY GAG EXCRETION

LD Cohort

In the LD cohort, serum ARSB increased from undetectable to 16% to 23% of the mean healthy value within 8 weeks after vector administration in participants 01-001 and 01-002. In participants 01-001 and 01-002, the mean (±SD) serum ARSB increase was sustained (485±82 and 351±75 pg/ml, respectively) up to weeks 54 and 55 respectively, when ERT was restarted in both participants due to increased (4.2- and 3.5-fold, respectively) urinary GAG concentrations compared with baseline (Fig. 1). In participant 01-003, serum ARSB remained undetectable at all time points and urinary GAG increased faster than the other two participants in the LD cohort (reaching concentrations that were threefold higher than baseline), thus requiring earlier reintroduction of ERT at week 27 after gene therapy (Fig. 1). On reintroduction of ERT, the urinary GAG concentration returned to baseline in all three participants. After reintroduction of ERT, serum ARSB measured at trough was higher than the corresponding pre–gene therapy values (Fig. 2A). In contrast, 5 to 6 days after ERT, serum ARSB was comparable to the corresponding pre–gene therapy values in participant 01-003, who did not show a detectable increase in serum ARSB after gene therapy (Fig. 2A). Despite participant 01-002 missing several ERT infusions during the Covid-19 pandemic, his urinary GAG excretion remained stable after 14- to 21-day intervals between ERT infusions and was lower than the corresponding post-ERT values before gene therapy (Fig. 2B).

ID Cohort

In the two participants in the ID cohort, serum ARSB increased to 16% to 19% of the mean healthy value, similar to the LD cohort. Mean (±SD) serum ARSB remained sustained (402±93 and 346±75 pg/ml) in participants 01-004 and 01-005, respectively (Fig. 1), until both participants resumed ERT because of increased urinary GAG (2.2- and 3-fold, respectively; Fig. 1). Upon resumption of ERT, urinary GAG concentrations returned to baseline in both participants. As observed for participants 01-001 and 01-002 in the LD cohort, serum ARSB was higher than the corresponding pre–gene therapy values, suggesting sustained ARSB hepatic expression up to months 15 and 32, the last time points available for participants 01-004 and 01-005, respectively (Fig. 2A). During the Covid-19 pandemic, participant 01-005 started an every-other-week ERT regimen that he kept for about 1.5 year, during which his urinary GAG and clinical conditions remained unchanged (Fig. 2B). Because of the lack of a dose-dependent increase in serum ARSB and increased urinary GAG, the third participant in this cohort was not infused and instead was transferred to the next (HD) cohort after approval from the competent regulatory authorities and endorsement by the DSMB.

HD Cohort

All participants who received the high vector dose showed sustained higher mean (±SD) serum ARSB (697±205, 690±95, 2,049±632, and 591±192 pg/ml for participants 01-006, 01-007, 01-008 and 01-009, respectively) compared with both LD and ID cohorts that corresponded to and plateaued at approximately 30% of the mean healthy value. Participants 01-006, 01-007, and 01-009 showed modest increases in urinary GAG (Fig. 1), which were below the average concentration of untreated patients with MPS VI (321 mg/g of creatinine).[23] Thus, they have remained without ERT for up to 104 weeks, the longest time point of observation in this cohort. Participant 01-008 had peak serum ARSB similar to the mean healthy value with urinary GAG excretion comparable (1.3-fold) to baseline. However, starting from week 49, participant 01-008 showed a gradual decrease in serum ARSB with increased urinary GAG excretion (Fig. 1), which prompted the recommendation to restart ERT at week 71. No increases in either serum aspartate transaminase (AST) or ALT or anti-ARSB antibodies were detected in participant 01-008 at weeks 49 and 71.

CLINICAL END POINTS

Overall, no relevant changes were observed in endurance, as evaluated by the 6-minute walk test and the 3-minute stair-climb test before and after gene therapy or reintroduction of ERT (Fig. S4). Participant 01-003 had undetectable serum ARSB and complained of fatigue from the first month after gene therapy. Participant 01-004 showed reduced performance in the 6-minute walk test, which was associated with worsening of her craniocervical spinal cord compression, and this was resolved with surgery (Fig. S4).

Pulmonary function, as measured by forced vital capacity (FVC; percent predicted) and forced expiratory volume in 1 second (FEV_1; percent predicted), was moderately reduced at baseline in participants in the LD and ID cohorts and was normal in participants in the HD cohort. Both FVC and FEV_1 did not change significantly after gene therapy (Fig. S5), except for two participants whose

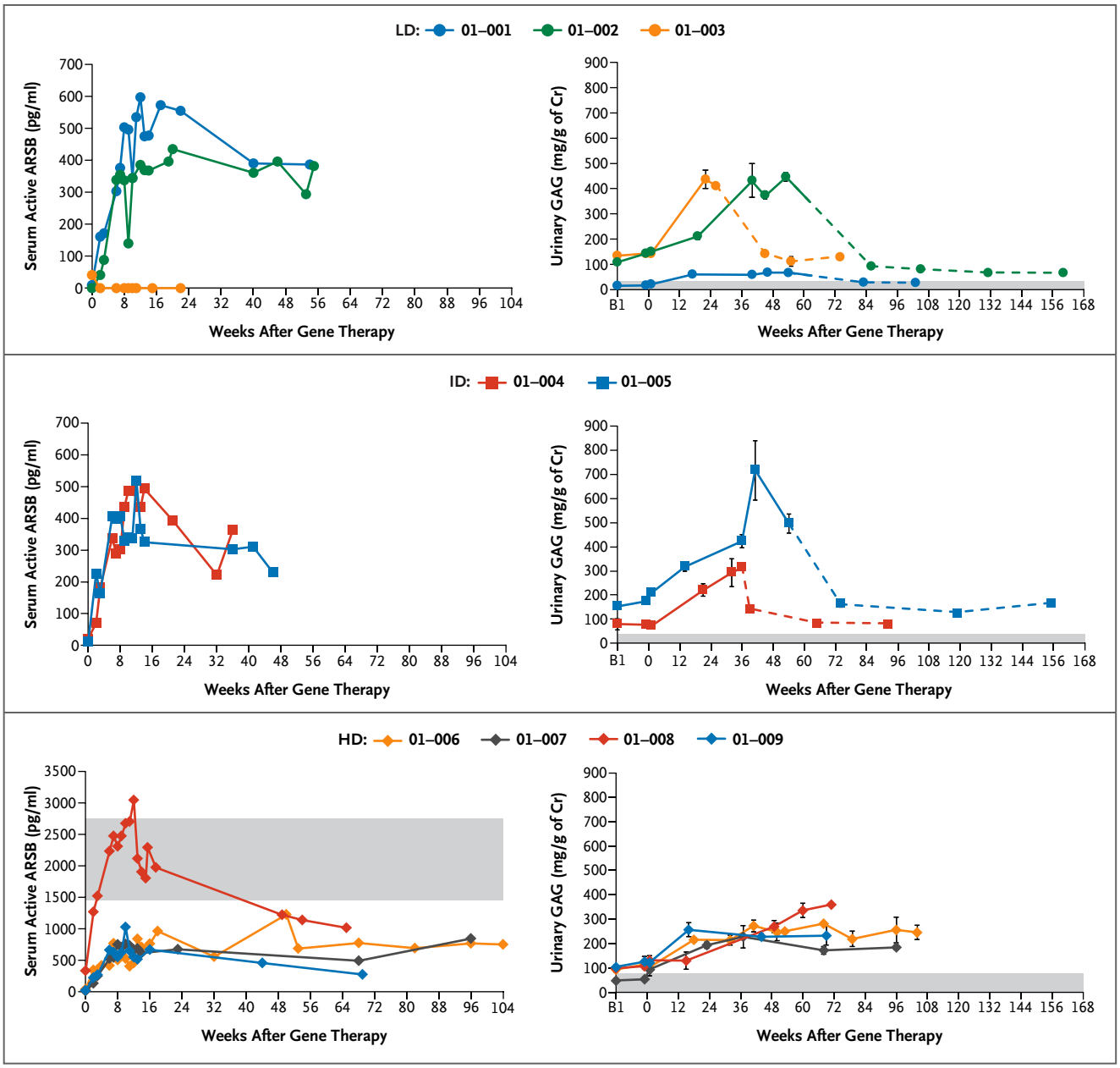

Figure 1. Serum Active Arylsulfatase B and Urinary Glycosaminoglycans.

In the Left Panels, serum active arylsulfatase B (ARSB) was measured before and after gene therapy in participants in the low-dose (LD), intermediate-dose (ID), and high-dose (HD) cohorts. (Note difference in the ordinate scale between HD and the LD and ID graphs.) Serum active ARSB was determined by comparing the enzymatic activity of the test samples to that of a standard curve made with recombinant human ARSB. For the LD and ID dose cohorts, only the values measured before restart of enzyme replacement therapy (ERT) are reported in the graph. The gray horizontal bar represents the mean (±SD) range of serum ARSB measured in healthy participants. In the Right Panels, urinary glycosaminoglycan (GAG) excretion was measured before and after gene therapy. Each value is the mean (±SE) of urinary GAG measured in two or three samples collected over 2 or 3 consecutive days. Measurement before gene therapy were performed at the Baseline 1 (B1) visit, when patients were under ERT and on day −2, −1, and 0 before gene therapy, when participants discontinued ERT 7 to 14 days before, as per clinical protocol. Dashed lines represent urinary GAG measured after ERT reintroduction. The gray horizontal bar represents the normal range of urinary GAG of healthy participants older than 13 years of age (8 to 31 mg/g of creatinine [Cr]) for the LD and ID cohorts, and 3 to 13 years of age (<80 mg/g of Cr) for the HD cohort. Patient identification numbers are shown in the key.

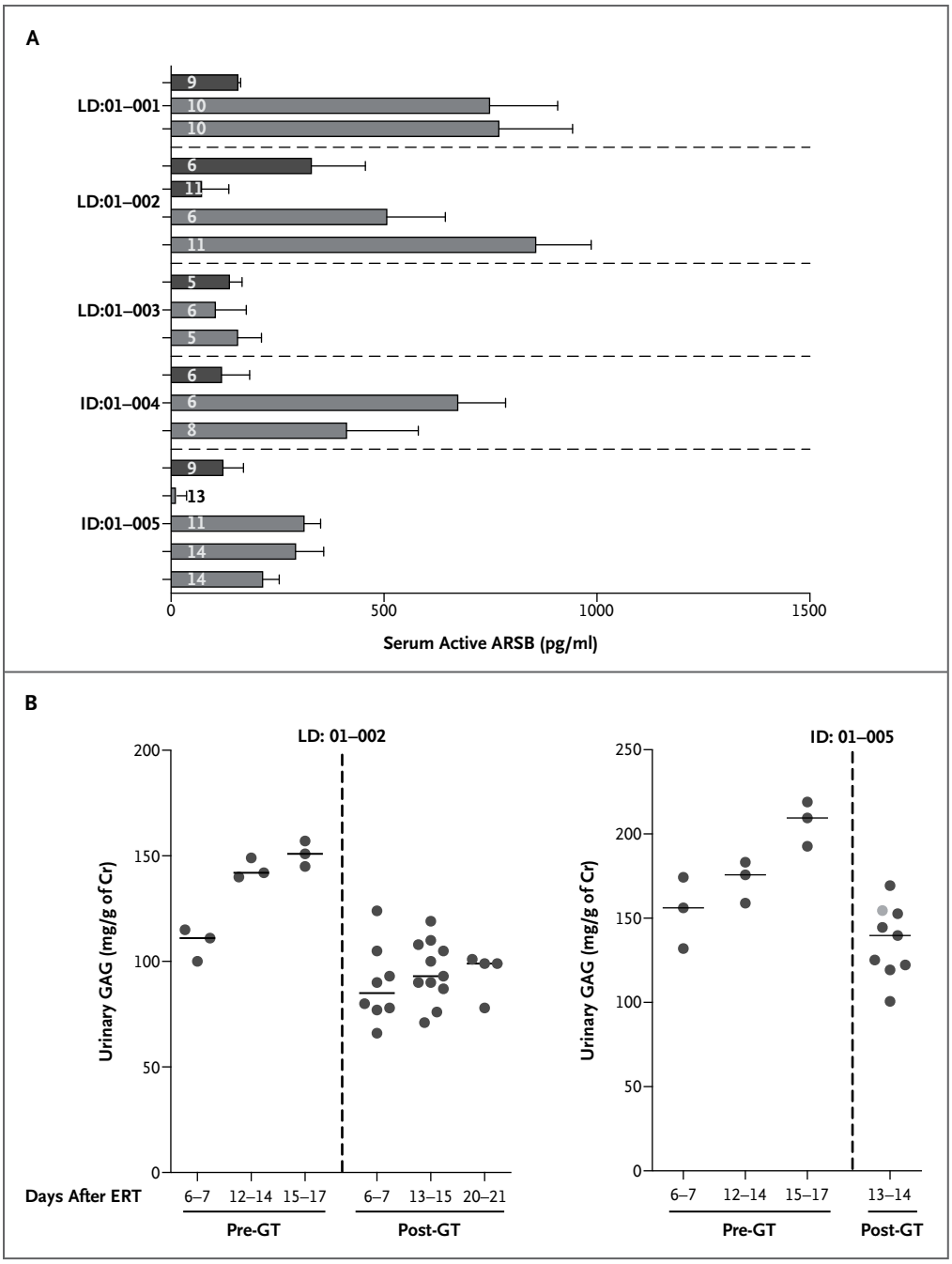

Figure 2. Serum Active Arylsulfatase B and Urinary Glycosaminoglycan Excretion in Participants Resuming Enzyme Replacement Therapy.

In Panel A, serum active arylsulfatase B (ARSB) was measured in participants resuming enzyme replacement therapy (ERT) several days after the last ERT infusion either before (black bars) or after (gray bars) gene therapy (GT). Numbers in bars refer to days after ERT administration. Each value is the mean (±SE) of technical replicates. In Panel B, urinary glycosaminoglycan (GAG) excretion was measured in participants 01-002 and 01-005 several days after the last ERT infusion either before or after gene therapy (on the left and on the right of the dashed line, respectively). Dots represent single values; the horizontal line is the mean. The gray dot, shown on the plot for participant 01-005, refers to GAG measured after 1.5 years of biweekly ERT regimen. Cr denotes creatinine, ID intermediate dose, and LD low dose.

performance decreased by 15 to 17 percentage points of predicted FVC.

All participants, except for one, had normal left ventricle ejection fraction measured by ultrasound at baseline (range, 55% to 65%) that remained unchanged during follow-up. Left ventricle end-diastolic interventricular septum and internal diameters, as well as aortic, mitral, and tricuspid valve leaflet thickness, also did not change significantly. One patient (01-003) developed congestive heart failure due to worsening of preexisting mitral and tricuspid insufficiency as determined by ultrasonography. He underwent mitral valve replacement and tricuspid commissuroplasty with resolution of his heart failure.

Liver and spleen longitudinal diameters remained stable in five participants in the LD and ID groups, whereas one participant (01-002, LD cohort) showed increased liver and spleen sizes compared with baseline that resolved upon ERT reintroduction. All participants in the HD cohort had normal liver and spleen sizes at baseline that remained within the normal range throughout the measurements (Fig. S6). No significant changes in (C)HAQ-DI scores were observed (Table S6).

Discussion

We studied the safety and efficacy of a single intravenous administration of AAV2/8.TBG.h*ARSB* in patients with MPS VI. We found that intravenous infusions of the vector did not result in adverse vector-related events in all nine participants, consistent with the nonclinical safety studies in mice that did not show toxicity up to the highest dose tested (2×10^{13} genome copies/kg, which is an half-log higher than the HD in our study), except for a transient increase in serum ALT (only in female mice) and thyroid epithelial hypertrophy.[20] Participants in the LD and ID cohorts showed serum ARSB of approximately 20% of mean healthy value but returned to ERT because of increased urinary GAG. Participants in the HD cohort had sustained serum ARSB of 30% to 100% of mean healthy value, and only modest increase in urinary GAG.

Previous clinical studies have showed a cytotoxic T lymphocyte-mediated immune response against transduced hepatocytes leading to liver inflammation that appear to be controlled by glucocorticoids.[24-28] Although serum ALT modestly increased in four of nine participants, the values measured remained within the reference range and all but one participant (01-001) showed reduced serum ALT after a brief treatment with a glucocorticoid. Cell-mediated immune responses against either the AAV8 capsid or the ARSB protein as well as serum cytokines and chemokines were not significantly different between participants, whether or not they had the transient elevations of serum ALT. Liver transaminases and peripheral blood mononuclear cells were monitored up to 14 weeks after gene therapy based on the hemophilia B gene therapy clinical study with AAV8.[24,25] However, a later cell-mediated immune response, as recently observed in hemophilia A participants receiving AAV5, cannot be ruled out.[26,29] Participant 01-008 in the HD cohort who had the highest expression of serum ARSB following gene therapy, showed gradual decline in serum ARSB between weeks 15 and 49 with concomitant increase in urinary GAG. Although participant 01-008 did not report any symptoms and she did not show signs of worsening of her underlying disease, ERT was recommended 1.4 years after gene therapy. Serum AST and ALT and the immune responses measured during the serum ARSB decline were all unremarkable. Although unlikely at this late time point after AAV infusion, a cell-mediated immune responses to AAV8 could not be ruled out because of limitation to obtain samples during the Covid-19 pandemic. Vector shedding lasted longer than other clinical studies using AAV8[25] but was similar to the AAV5 hemophilia A clinical study.[26]

In eight of the nine participants, serum ARSB increased above baseline from week 2 after vector infusion, plateaued between 16% and 100% of mean normal values by 6 to 8 weeks, and remained stable for up to 131 weeks (approximately 32 months) post-administration. Therefore, a single administration of AAV8 provided sustained hepatic expression of the therapeutic protein, consistent with the findings in individuals with hemophilia A and B.[24-27] While in the hemophilia studies patients undergoing gene therapy were typically adults and male, in the present study male and female patients were treated, and eight of the nine participants were younger than 18 years. With three participants 10 years of age and one 5 years of age, we contend that patients included in this study are among the youngest participants in a liver-directed gene therapy clinical study. Participants of pediatric age have been enrolled in AAV studies in which direct measurement of transgene expression over time could not be performed.[28] In contrast, the present study involves an AAV-encoded secreted protein and addresses duration of transgene expression in growing individuals. Sustained

transgene expression suggests that the rate of hepatocyte proliferation at the ages of enrolled patients does not cause significant dilution of the AAV episomal genome, at least during an observation period of 32 months. Further follow-up is needed to establish the ultimate duration and stability of transgene expression in young individuals, as well as the potential risks of insertional mutagenesis.

In participants who received the low and intermediate vector doses, sustained serum ARSB of 16% to 23% of the mean healthy value was unable to prevent an increase in urinary GAG concentrations that rose to values detected in patients with naïve untreated MPS VI,[23] thus mandating the reintroduction of ERT. ERT was also reintroduced in participant 01-001 who showed only a slight increase in urinary GAG concentrations and suffered a milder form of the disease.

Of note, during the Covid-19 pandemic, despite missing one or two infusions after weekly galsulfase had been reintroduced, two participants showed unchanged urinary GAG during these 14- to 21-day intervals, with concentrations that were below those measured before gene therapy. Importantly, participant 01-005, who received ERT every other week for 1.5 years, had stable urinary GAGs and clinical conditions. These data suggest that although vector-derived ARSB expression was not sufficient to maintain urinary GAG concentrations at baseline in the LD and ID cohorts, the intervention may reduce the requirement for enzyme infusions, as previously reported in MPS VI mice[19] and in patients with hemophilia B who achieved low levels of factor IX by gene therapy that allowed a reduced frequency of clotting factor infusions.[25] Although late ERT reintroduction poses the risk of development of neutralizing antibodies,[30] liver gene therapy may mitigate this risk by inducing immunotolerance to the transgene product as observed in small and large animals.[31-33] Further clinical study is needed to determine whether this occurs.

The increased serum ARSB after the high vector dose was associated with a modest increase in urinary GAG in three of four participants who did not require ERT reintroduction up to 2 years after gene therapy, a time point at which the other patients in both the LD and ID cohorts had returned to ERT.

A control group was not included in this study, but the stabilization of urinary GAG in participants in the HD cohort suggests that liver-directed gene therapy might prevent disease progression, consistent with the results in MPS VI animal models, albeit slightly lower AAV doses were more effective in animals.[16,17] Nevertheless, we speculate that a higher vector dose (up to 1×10^{13} genome copies/kg) might provide improved efficacy, and a longer follow-up in more patients recruited to a phase 3 study will be needed to determine whether this form of gene therapy can be successful in mitigating or arresting disease progression.

In conclusion, a single intravenous administration of AAV2/8.TBG.*hARSB* in nine patients was not associated with immediate adverse reactions and was associated with changes in expression of serum ARSB and a mild increase in urinary GAG only in the high dose-group, thus supporting effective liver gene transfer. Further follow-up of these patients and larger and longer clinical trials are needed to establish the potential clinical utility of this treatment.

Disclosures

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