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Hematopoietic Stem- and Progenitor-Cell Gene Therapy for Hurler Syndrome

B. Gentner, F. Tucci, S. Galimberti, F. Fumagalli, M. De Pellegrin, P. Silvani, C. Camesasca, S. Pontesilli, S. Darin, F. Ciotti, M. Sarzana, G. Consiglieri, C. Filisetti, G. Forni, L. Passerini, D. Tomasoni, D. Cesana, A. Calabria, G. Spinozzi, M.-P. Cicalese, V. Calbi, M. Migliavacca, F. Barzaghi, F. Ferrua, V. Gallo, S. Miglietta, E. Zonari, P.S. Cheruku, C. Forni, M. Facchini, A. Corti, M. Gabaldo, S. Zancan, S. Gasperini, A. Rovelli, J.-J. Boelens, S.A. Jones, R. Wynn, C. Baldoli, E. Montini, S. Gregori, F. Ciceri, M.G. Valsecchi, G. la Marca, R. Parini, L. Naldini, A. Aiuti, and M.-E. Bernardo, for the MPSI Study Group*

ABSTRACT

BACKGROUND

Allogeneic hematopoietic stem-cell transplantation is the standard of care for Hurler syndrome (mucopolysaccharidosis type I, Hurler variant [MPSIH]). However, this treatment is only partially curative and is associated with complications.

METHODS

We are conducting an ongoing study involving eight children with MPSIH. At enrollment, the children lacked a suitable allogeneic donor and had a Developmental Quotient or Intelligence Quotient score above 70 (i.e., none had moderate or severe cognitive impairment). The children received autologous hematopoietic stem and progenitor cells (HSPCs) transduced ex vivo with an α-L-iduronidase (IDUA)–encoding lentiviral vector after myeloablative conditioning. Safety and correction of blood IDUA activity up to supraphysiologic levels were the primary end points. Clearance of lysosomal storage material as well as skeletal and neurophysiological development were assessed as secondary and exploratory end points. The planned duration of the study is 5 years.

RESULTS

We now report interim results. The children's mean (±SD) age at the time of HSPC gene therapy was 1.9±0.5 years. At a median follow-up of 2.10 years, the procedure had a safety profile similar to that known for autologous hematopoietic stem-cell transplantation. All the patients showed prompt and sustained engraftment of gene-corrected cells and had supraphysiologic blood IDUA activity within a month, which was maintained up to the latest follow-up. Urinary glycosaminoglycan (GAG) excretion decreased steeply, reaching normal levels at 12 months in four of five patients who could be evaluated. Previously undetectable levels of IDUA activity in the cerebrospinal fluid became detectable after gene therapy and were associated with local clearance of GAGs. Patients showed stable cognitive performance, stable motor skills corresponding to continued motor development, improved or stable findings on magnetic resonance imaging of the brain and spine, reduced joint stiffness, and normal growth in line with World Health Organization growth charts.

CONCLUSIONS

The delivery of HSPC gene therapy in patients with MPSIH resulted in extensive metabolic correction in peripheral tissues and the central nervous system. (Funded by Fondazione Telethon and others; ClinicalTrials.gov number, NCT03488394; EudraCT number, 2017-002430-23.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Aiuti can be contacted at aiuti.alessandro@hsr.it or at the San Raffaele Telethon Institute for Gene Therapy, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy.

*The members of the MPSI Study Group are listed in the Supplementary Appendix, available at NEJM.org.

Drs. Tucci and Galimberti and Drs. Aiuti and Bernardo contributed equally to this article.

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Hurler syndrome (mucopolysaccharidosis type I, Hurler variant [MPSIH]) is the most severe phenotype of mucopolysaccharidosis type I,[1,2] a rare autosomal recessive lysosomal storage disease caused by loss-of-function variants of the *IDUA* gene, which encodes the enzyme α-L-iduronidase (IDUA); this loss of function leads to glycosaminoglycan (GAG) accumulation throughout peripheral organs and the central nervous system (CNS). Somatic clinical manifestations include coarse facial features, dysostosis multiplex, hepatosplenomegaly, hearing loss, visual impairment, upper airway obstruction, valvular heart disease, restrictive lung disease, brain atrophy, and spinal cord compression. Neurocognitive regression develops progressively in patients with MPSIH, and they die within their first decade of life.[3] Enzyme-replacement therapy (ERT) with laronidase reduces GAG accumulation and alleviates some somatic features. However, skeletal and CNS manifestations are not controlled,[4] and anti-IDUA antibodies that develop in most patients may limit the efficacy of ERT.[5,6]

Allogeneic hematopoietic stem-cell transplantation with pretransplantation or peritransplantation ERT is the standard of care for patients with MPSIH.[7,8] Treated patients have high rates of overall survival, especially when treated at a relatively young age and when cord-blood hematopoietic stem and progenitor cells (HSPCs) are used.[9,10] Donor-derived hematopoietic cells constitute a stable endogenous source of the enzyme released into the systemic circulation and locally in the tissues, from tissue-resident myeloid cells replaced after transplantation, which may cross-correct neighboring nonhematopoietic cells. The latter mechanism may also support enzyme delivery into the CNS, lessening neurocognitive impairment with hematopoietic stem-cell transplantation as compared with ERT.[11] However, cognitive and skeletal abnormalities persist and progress over time after allogeneic hematopoietic stem-cell transplantation, severely affecting patients' quality of life.[12]

We have designed a new strategy based on genetic engineering of autologous HSPCs to drive supranormal IDUA activity that is distributed to affected tissues through the turnover of resident hematopoietic cells. In a mouse model of mucopolysaccharidosis type I, we found that disease manifestations, including the neurologic and skeletal defects, were corrected with lentiviral vector–based HSPC gene therapy with superior proficiency as compared with hematopoietic stem-cell transplantation from wild-type donors, probably owing to higher IDUA expression from the engineered cassette with respect to the endogenous locus.[13] Here we report the interim safety and efficacy results of eight patients with MPSIH treated with autologous HSPCs genetically modified to overexpress human IDUA, with follow-up ranging from 1.46 to 2.90 years.

Methods

Study Design and Oversight

This is a phase 1–2, nonrandomized, single-center study involving eight patients with MPSIH who are undergoing HSPC gene therapy with *IDUA* lentiviral vector. The planned duration of the study is 5 years. Furthermore, the patients will be followed for at least 15 years in a dedicated long-term follow-up study. The study was approved by the Italian Regulatory Authority and the Ethical Committee of the San Raffaele Scientific Institute and was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The San Raffaele Hospital and Fondazione Telethon are the study promoter and financial sponsor, respectively. On May 24, 2019, Orchard Therapeutics was granted a license for further development of gene therapy for MPSIH. The San Raffaele Telethon Institute for Gene Therapy was responsible for the design of the study, the collection and analysis of the data, the writing of the manuscript, and the decision to submit the manuscript for publication. All the authors had access to the complete data and contributed to data interpretation and manuscript preparation. The authors vouch for the completeness and accuracy of the data and for the fidelity of the study to the protocol, available with the full text of this article at NEJM.org.

Patients and Procedures

After informed consent was obtained from parents, eight children with MPSIH were treated from July 2018 through December 2019. All the children met the inclusion criteria and did not meet any of the exclusion criteria (Table S1 in the Supplementary Appendix, available at NEJM.org). In particular, they lacked a suitable allogeneic donor and had a Developmental Quotient (DQ)

or Intelligence Quotient (IQ) score of more than 70. (Scores of <55 indicate severe cognitive impairment; scores of 55 to 70, moderate cognitive impairment; scores of 71 to 85, mild cognitive impairment; scores of 86 to 110, normal cognitive function; and scores of >110, above-normal cognitive function.) Autologous HSPCs were collected by leukapheresis on 2 consecutive days after mobilization from the bone marrow niche with granulocyte colony-stimulating factor (5 to 10 μg per kilogram of body weight per day for 3-5 days) and plerixafor (0.24 to 0.40 mg per kilogram on the days of leukapheresis) to reach a drug-product dose of 4×10^6 to 35×10^6 CD34+ cells per kilogram and a cryopreserved backup of at least 3×10^6 CD34+ cells per kilogram. CD34+-enriched cells were transduced with clinical-grade lentiviral vector encoding human *IDUA* complementary DNA under the control of the human phosphoglycerate kinase 1 gene promoter with the use of an abbreviated, 36-hour, ex vivo manufacturing protocol including prostaglandin E_2 and a single round of transduction,[14] and the cells were cryopreserved. For the purpose of this interim analysis, the infusion day was designated as day 0 and the first day after infusion as day 1. The conditioning regimen was rituximab (375 mg per square meter of body-surface area on day −15), busulfan (from day −5 to day −2, single daily intravenous dose starting with 80 mg per square meter, then adjusted to a myeloablative total area-under-the-curve target of 85,000 μg×hours per liter based on daily therapeutic drug monitoring), and fludarabine (40 mg per square meter intravenously from day −5 to −2). The drug product was infused intravenously through a central venous catheter (Fig. S1). During hospitalization, patients remained in an isolation unit and received supportive therapy according to local standards.

SAFETY AND EFFICACY END POINTS

The primary safety end points include overall survival, hematologic engraftment by day 45, short- and long-term safety of drug-product infusion, and adverse-event monitoring. The primary efficacy end point is blood IDUA activity (up to supraphysiologic levels) at 1 year after treatment. Secondary end points include anti-IDUA antibody immune response (safety) and engraftment of transduced cells at levels of 30% or more, normalization of urinary GAGs, and

Table 1. Characteristics of the Patients at Baseline and Drug-Product Details.*

Patient No.	Race or Ethnic Group†	Sex	Age at Time of Gene Therapy	*IDUA* Gene Variant	DQ or IQ Score‡	CD34+ Dose	VCN in Drug Product	Transduced CFU	Time since Gene Therapy (latest visit)
			yr			*×10⁻⁶ cells/kg*	*copies/cell*	*%*	
1	White	M	1.97	c.603C→G/c.603C→G	75	24	2.1	>80	2.90 yr (2-yr vist)
2	Arabic Druze	M	1.15	c.192C→A/c.192C→A	100	14	5.2	>80	2.40 yr (2-yr visit)
3	White	F	1.95	c.208C→T/c.208C→T	75	18	2.3	>80	2.13 yr (2-yr visit)
4	White	M	1.21	c.208C→T/c.208C→T	95	29	1.0	66	2.07 yr (2-yr visit)
5	Black	M	2.84	c.1205G→A/c.1409delT	77	13	1.3	>80	2.18 yr (2-yr visit)§
6	White	M	2.13	c.1205G→A/c.979G→C	85	31	1.1	65	1.96 yr (1.5-yr visit)
7	Black	M	1.71	c.923_926delTGCC/c.923_926delTGCC	80	21	3.4	>80	1.73 yr (1.5-yr visit)
8	Persian	F	1.97	c.656G→A/c.1104_1105delCT	90	20	3.4	>80	1.46 yr (1-yr visit)

* CFU denotes colony-forming units, IDUA α-L-iduronidase, and VCN vector copy number.
† Race or ethnic group was reported by the parents of the patients.
‡ The Developmental Quotient (DQ) or Intelligence Quotient (IQ) score (cognitive score) was assessed by means of the Bayley Scales of Infant and Toddler Development, Third Edition, or the Wechsler Preschool and Primary Scale of Intelligence, Third Edition. Scores of less than 55 indicate severe cognitive impairment; scores of 55 to 70, moderate cognitive impairment; scores of 71 to 85, mild cognitive impairment; scores of 86 to 110, normal cognitive function; and scores of more than 110, above-normal cognitive function.
§ The 2-year visit was a remote visit.

growth velocity (efficacy) at 1 and 3 years after treatment. Exploratory end points include motor function as assessed according to the Peabody Developmental Motor Scales, Second Edition (for details, see the Supplementary Appendix). Study end points are summarized in Table S2. Owing to the severe acute respiratory syndrome coronavirus 2 pandemic, some follow-up visits had to be performed remotely with the support of local health care providers, without the possibility to assess all secondary end points.

STATISTICAL ANALYSIS

The sample size for the study was determined on the basis of demonstration of supraphysiologic IDUA activity (above the 97.5th percentile of values in healthy children) with 80% power with the use of a one-sided 2.5% significance level. The planned interim analysis that is reported here is descriptive, and no formal statistical testing was performed. Results are presented as mean and standard deviation or median and range as appropriate. Details are provided in the Supplementary Appendix.

RESULTS

PATIENT AND GRAFT CHARACTERISTICS

The characteristics of the patients at baseline and drug-product details are reported in Table 1. All eight patients with MPSIH (six boys and two girls; mean [±SD] age at the time of gene therapy, 1.9±0.5 years) had biallelic *IDUA* gene variants with known pathogenic potential, IDUA enzyme deficiency in the blood, increased urinary excretion of heparan sulfate and dermatan sulfate despite ERT administration, and a spectrum of clinical manifestations typical for MPSIH (Table S3). A high number of autologous HSPCs (mean [±SD], 44.5±10.9×10^6 CD34+ cells per kilogram) were collected after mobilization. CD34+-enriched cells were transduced with *IDUA* lentiviral vector and cryopreserved. The mean drug product CD34+ cell dose was 20.9±6.4×10^6 per kilogram, with a median vector copy number of 2.2 per genome (range, 1.0 to 5.2).

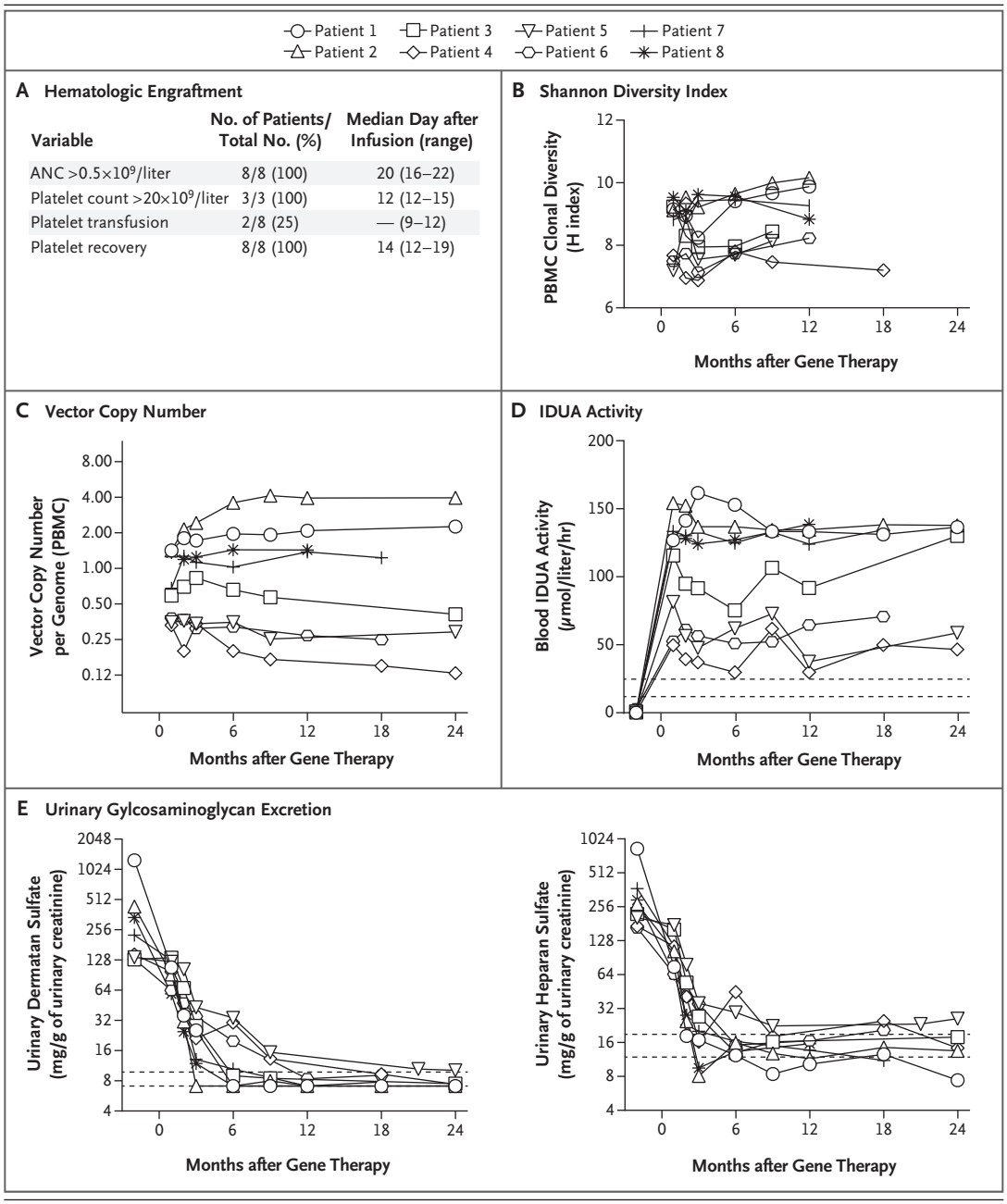
SAFETY

At the time of this writing, all the patients are alive and appear well after a median follow-up of 2.10 years (range, 1.46 to 2.90) and a total of 16.84 person-years of observation. Hematologic engraftment was rapid and consistent in all the patients (Fig. 1A and Fig. S2A, S2B, and S2C), with neutrophil recovery occurring at a median of day 20 (range, 16 to 22). Three of eight patients had grade 4 thrombocytopenia, each on a single day; however, all the patients had an early, spontaneous platelet recovery (median, day 14; range, 12 to 19). Lymphocyte counts recovered 1 month after treatment (Fig. S2D). Transient T-cell depletion due to fludarabine conditioning did not hamper maintenance of immunologic T-cell memory to pathogens, as shown by measurement of T-cell response to cytomegalovirus before and after HSPC gene therapy (Fig. S2E). We did not observe viral reactivations in the early post-transplantion period, and given the autologous nature of the treatment, graft-versus-host disease developed in no patients.

A total of 19 serious adverse events have been reported, some of which (7 events [37%]) were related to known complications of MPSIH[15-18] and were already present before HSPC gene therapy (Table S4). An acute hypersensitivity re-

Figure 1 (facing page). Hematopoietic Reconstitution with Genetically Engineered Cells and Biochemical Correction.

Hematologic recovery, as shown in **Panel A**, was defined as the first of at least 3 consecutive days with an absolute neutrophil count (ANC) of more than 0.5×10^9 per liter and a platelet count of more than 20×10^9 per liter. Platelet recovery was defined as a platelet count of more than 20×10^9 per liter and spontaneously rising platelet counts on at least 3 consecutive days. Only two patients received prophylactic platelet transfusions, one of which occurred after a head trauma. In **Panel B**, the Shannon diversity index (H index), as derived from the unique lentiviral integrations detected in peripheral-blood mononuclear cells (PBMCs), is shown for the entire cohort over time. This method measures the entropy (diversity) for each time point of a vector-marked cell population by accounting for the number of distinct integration sites (richness) as well as their abundance (amount of genomes per integration site, evenness). **Panel C** shows the vector copy number in PBMCs over time. **Panel D** shows α-L-iduronidase (IDUA) activity measured on dried blood spots over time. The upper dashed line represents the 97.5th percentile of age-matched, healthy children; the lower dashed line represents the average of age-matched, healthy children. **Panel E** shows urinary excretion of dermatan sulfate (left) and heparan sulfate (right). The upper dashed line represents the upper limit of normal in children 1 to 3 years of age; the lower dashed line represents the upper limit of normal in children older than 3 years of age.



action that occurred in Patient 5 on day 12 after HSPC gene therapy and concomitantly to a first administration of vancomycin was considered by the investigators to be probably related to HSPC gene therapy. (Details on specific immunologic testing are provided in Fig. S3.) The event resolved promptly after treatment with antihistamines, intravenous fluids, and glucocorticoids, with no sequelae. The incidence of adverse events is summarized in Table S5.

The lentiviral vector integration profile that was retrieved from peripheral blood and bone marrow collected over multiple time points (Fig. S4A) was consistent with those in other studies of lentiviral vector hematopoietic stem-cell gene therapy.[19-21] All the patients had a stable and highly polyclonal repertoire, with no dominant integration sites expanding or persisting over time (Fig. 1B and Figs. S5 and S6). Moreover, common-insertion-site analysis did not highlight

any significant enrichment for oncogenes or tumor-suppressor genes (Fig. S4B).[19-21]

Seven of eight treated patients received ERT before HSPC gene therapy for a median of 5.1 months (range, 4.4 to 26.7). At baseline, anti-IDUA IgG antibodies were detected in five of these seven patients (Table S6); these antibodies disappeared in the first 3 months after HSPC gene therapy (percentage of patients negative for anti-IDUA IgG at baseline, 38%; at day 30, 75%; and at day 90 up to last follow-up, 100%).

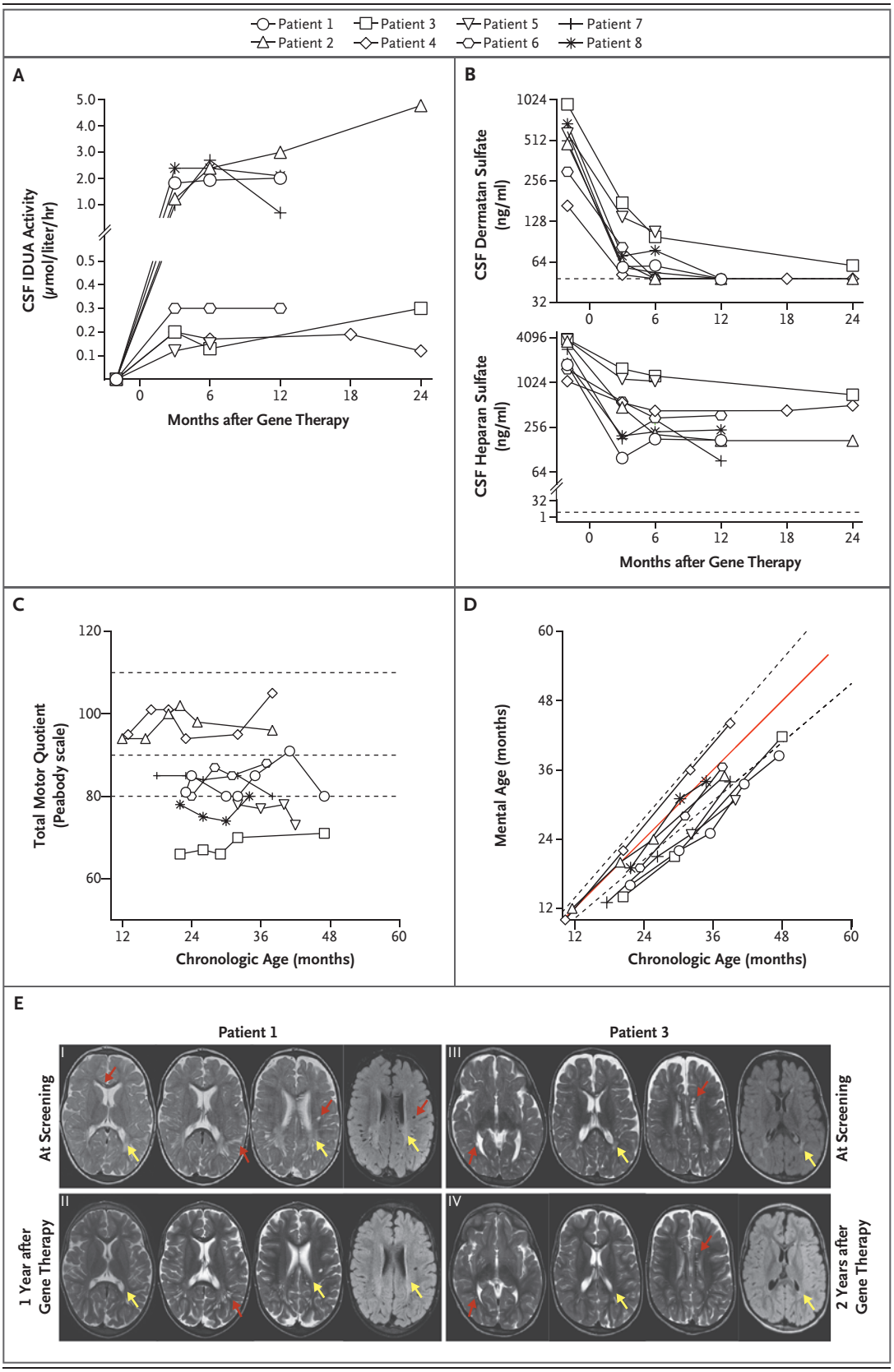
GENE MARKING AND BIOLOGIC OUTCOMES

In vivo gene marking with *IDUA* lentiviral vector stabilized after 6 months, reaching a median vector copy number of 0.98 per genome (range, 0.17 to 3.95) in peripheral-blood mononuclear cells (PBMCs) at 9 to 12 months after HSPC gene therapy (Fig. 1C). Five of eight patients (62%) had long-term engraftment levels of transduced PBMCs above 30%. Gene marking was similar in multiple hematopoietic lineages from peripheral blood and bone marrow (Fig. S7), findings consistent with transduction of primitive, multipotent HSPCs.

Concomitant with hematologic engraftment, blood IDUA activity reached supraphysiologic levels as early as 30 days after HSPC gene therapy in all the patients (Fig. 1D). At 12 months, eight of eight patients (100%) had supraphysiologic blood IDUA activity (median, 108.0 μmol per liter per hour; range, 30.0 to 138.6), above the 97.5th percentile (24.8 μmol per liter per hour) of age-matched (i.e., 6 months to 6 years), healthy children (median, 11.1 μmol per liter per hour; range, 3.8 to 35.0) (see the Methods section in the Supplementary Appendix). Cellular IDUA activity in myeloid populations and PBMCs confirmed enzyme hyperproduction by transduced hematopoietic cells, which normalized IDUA activity in the plasma (Fig. S8). All the patients had a steep decline in pathologic GAG excretion in the urine, which reached normal or near-normal values and remained stable at last follow-up (Fig. 1E). Seven of eight patients (88%) and six of eight patients (75%) had normalization of urinary heparan sulfate and dermatan sulfate levels, respectively, some as early as day 90 after treatment. An external group of patients with MPSIH who underwent allogeneic hematopoietic stem-cell transplantation (Table S7) showed low-normal blood IDUA activity (19 patients; follow-up range, 1 month to 20 years) and above-normal urinary GAG excretion (7 patients; follow-up range, 9 months to 2 years), which suggests a less complete metabolic correction (Fig. S9).

Figure 2 (facing page). CSF Biochemical Data, Imaging, and Early Neurologic Outcomes.

Panel A shows IDUA activity in cerebrospinal fluid (CSF) for each of the eight patients at baseline and after treatment (at 6, 12, and 24 months). For Patient 4, who missed the 12-month follow-up visit, data for the 6-month, 18-month, and 24-month follow-up visits are reported. **Panel B** shows heparan sulfate and dermatan sulfate levels in CSF for each of the eight patients at baseline and after treatment (at 6, 12, and 24 months). For Patient 4, who missed the 12-month follow-up visit, data for the 6-month, 18-month, and 24-month follow-up visits are reported. The dashed line indicates the lower limit of detection. **Panel C** shows changes in the total motor quotient (TMQ) over time for each of the eight patients according to chronologic age. TMQs were assessed at baseline and every 6 months after treatment with the use of the Peabody Developmental Motor Scales, Second Edition (PDMS-2). The upper and middle dashed lines represent the normal range of TMQs in the PDMS-2 for healthy children, whereas the lower dashed line represents the low average of normal for healthy children. Scores were assigned by two physical therapists trained in the use of the PDMS-2. **Panel D** shows cognitive age-equivalent performance over time for each of the eight patients as measured with the use of the Bayley Scales of Infant and Toddler Development, Third Edition, and the Wechsler Preschool and Primary Scale of Intelligence, Third Edition, according to the child's chronologic age. The red line indicates the average; the dashed lines indicate the upper and lower limits of the normal range (1 SD). **Panel E** shows magnetic resonance imaging of the brain in two patients (Patients 1 and 3), with T2 and fluid-attenuated inversion recovery (FLAIR) axial images showing typical findings of patients with mucopolysaccharidosis type I, Hurler variant (MPSIH). Subpanel I shows mild ventriculomegaly; enlarged perivascular spaces (PVSs) (red arrows), widespread in posterior white matter and corpus callosum characterized by cystic appearance with cerebrospinal fluid signal intensity; and periventricular white-matter abnormalities (yellow arrows) characterized by high signal intensity, both on T2 and FLAIR images, at baseline. Subpanel II shows normal ventricular dimensions, improvement of white-matter abnormalities, and a reduction in the size of cystic appearance of PVSs at 12 months after hematopoietic stem- and progenitor-cell (HSPC) gene therapy. Subpanel III shows enlarged PVSs, widespread in the posterior white matter and corpus callosum; periventricular white-matter abnormalities; and moderate enlargement of frontal and interhemispheric subarachnoid spaces at baseline. Subpanel IV shows regression of white-matter abnormalities, a reduction in the size of cystic appearance of PVSs, and stable enlargement of frontal and interhemispheric subarachnoid spaces at 24 months after HSPC gene therapy.



EARLY NEUROLOGIC OUTCOMES

Previously undetectable levels of IDUA in the cerebrospinal fluid (CSF) at baseline were detectable after HSPC gene therapy in all the patients, starting from the first available time point at 3 months after treatment and persisting up to the latest follow-up (median, 0.50 μmol per liter per hour; range, 0.12 to 4.78) (Fig. 2A). GAG levels in the CSF declined with treatment (median at baseline: heparan sulfate, 2378 ng per milliliter [range, 1070 to 3950]; dermatan sulfate, 542 ng per milliliter [range, 168 to 947]; at 6 months: heparan sulfate, 335 ng per milliliter [range, 178 to 1255]; dermatan sulfate, 57 ng per milliliter [range, <48 to 107]; and at last follow-up: heparan sulfate, 304 ng per milliliter [range, 91 to 1070]; dermatan sulfate, 48 ng per milliliter [range, <48 to 107]). These findings suggest a rapid and profound metabolic correction of the CNS (Fig. 2B).

All the patients progressively acquired motor skills, as shown by the stability of Total, Gross, and Fine Motor Quotient scores[22] over time (Fig. 2C and Fig. S10), with six patients having total motor performance within normal limits or in the low average of normal and two being below the normal range at last follow-up. Although our observation period is limited, progressive gain of cognitive and language skills was observed throughout the follow-up period, regardless of whether the DQ or IQ score at baseline was normal or below average (Fig. 2D and Fig. S11). The four patients who had cognitive performance within the normal range at baseline (DQ or IQ score, ≥85) (Table 1) continue to track in the normal range.

A qualitative comparison of magnetic resonance imaging (MRI) of the brain that was performed at 12 and 24 months after HSPC gene therapy showed reductions in white-matter and perivascular-space abnormalities (Fig. 2E) as compared with baseline in three of five patients and two of three patients who could be evaluated, respectively. Alterations were stable in the remaining children.

GROWTH AND OTHER SYSTEMIC CLINICAL OUTCOMES

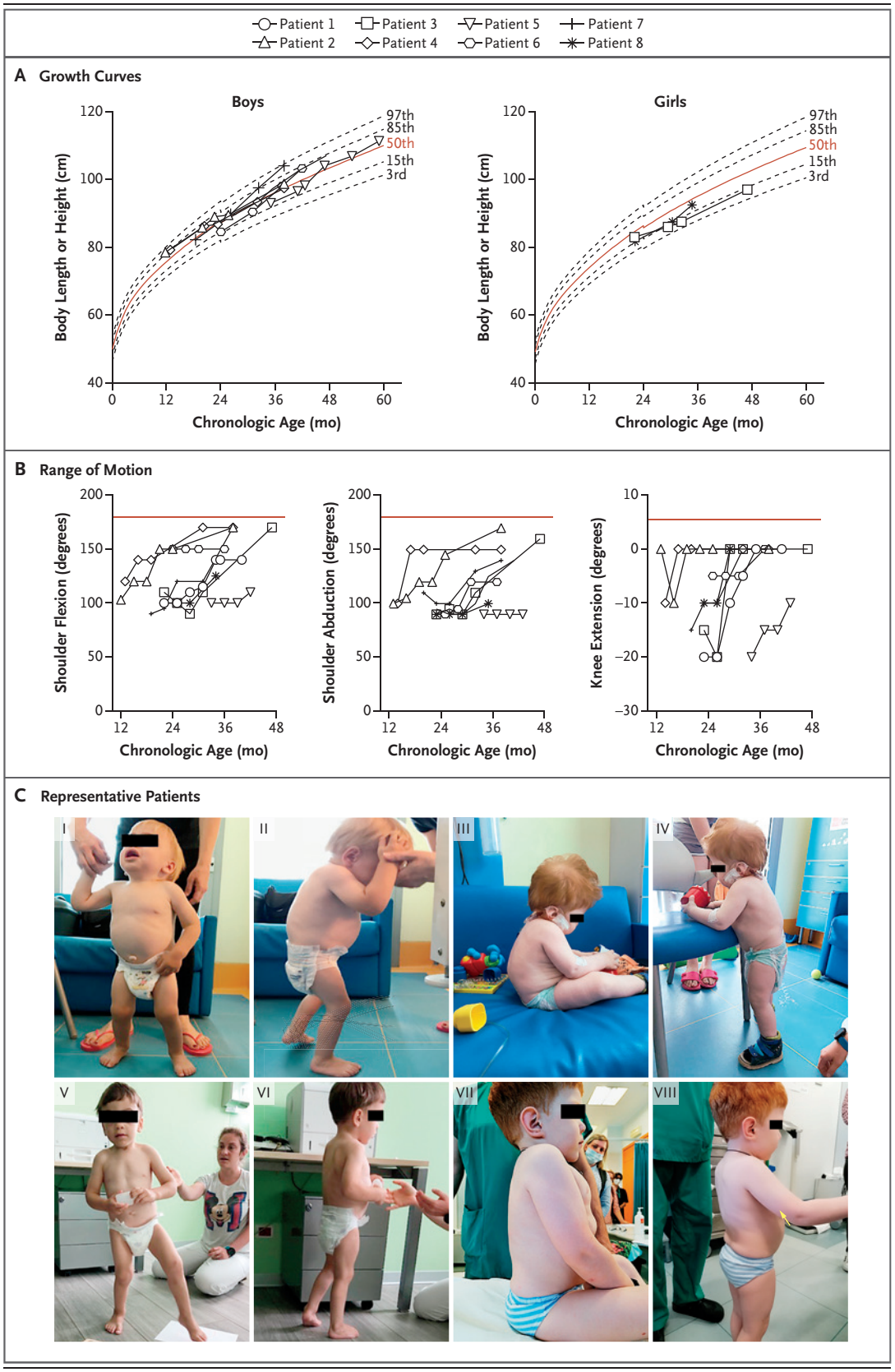
Longitudinal growth was measured as a surrogate for musculoskeletal disease manifestations. Although follow-up is limited, patients continue to track along their baseline World Health Organization growth percentiles (Fig. 3A). Median height percentiles at baseline, 1-year follow-up, and 2-year follow-up were 42 (range, 14 to 90; median standard deviation score, −0.21; eight patients), 61 (range, 33 to 86; median standard deviation score, 0.27; five patients), and 63 (range, 9 to 83; median standard deviation score, 0.34; five patients), respectively.

Range of motion was measured at the shoulder and knee joints before and after HSPC gene therapy. We observed progressive improvements in the range of motion of each joint; the improvements became apparent from 6 to 9 months after HSPC gene therapy (Fig. 3B and Fig. S12).

MRI of the spine at 6, 12, and 24 months of follow-up showed mild improvement or stability of the typical MRI features — that is, cervico-

Figure 3 (facing page). Early Skeletal Outcomes and Typical Disease Features in Eight Patients with MPSIH before and after HSPC Gene Therapy.

Panel A shows growth curves in six male and two female patients. Growth standards are based on length for 0 to 24 months of age and on height for more than 24 months of age, according to World Health Organization charts. **Panel B** shows passive range of motion of shoulder flexion, shoulder abduction, and knee extension as measured by an experienced orthopedist, together with two physical therapists, with a goniometer. Data were recorded according to the SFTR (sagittal, frontal, transverse, rotation) method. The red solid line represents the physiologic range of motion as reported previously by Soucie et al.[23] and Marucha et al.[24] **Panel C** shows photographs of two representative patients, Patients 1 and 4, before and after HSPC gene therapy; these photographs highlight some of the typical clinical manifestations of MPSIH. Subpanels I and II show Patient 1 before HSPC gene therapy, at 12 months of age. Coarse facial features, an enlarged abdomen due to liver and spleen involvement, umbilical hernia, knee contracture, and thoracolumbar kyphosis are evident. Patient 1 did not receive enzyme-replacement therapy (ERT) before HSPC gene therapy. Subpanels III and IV show Patient 4 before HSPC gene therapy, at 14 months of age. Thoracolumbar kyphosis and knee contracture are noticeable, despite the younger age at treatment and the early administration of ERT. Subpanels V and VI show Patient 1 at 12 months after HSPC gene therapy, at 36 months of age. The photographs show a milder disease phenotype with less evident facial features, a less prominent abdomen, improvement in knee extension, and a reduction in thoracolumbar kyphosis. Subpanels VII and VIII show Patient 4 at 24 months after HSPC gene therapy, at 38 months of age. This patient also shows an improvement in the clinical phenotype, with less evident facial features and a reduction in joint stiffness and thoracolumbar kyphosis. Parents consented to the use of photographs.



thoracic and thoracolumbar kyphosis and foramen magnum and spinal canal stenosis. (Representative MRI sequences are provided in Fig. S13.)

All the patients had liver and spleen sizes within normal ranges for age at baseline; these measurements remained within normal limits at the last follow-up (data not shown). Other typical clinical manifestations (e.g., coarse facial features, upper airway obstruction, hearing loss, and corneal opacity) that were evident at the time of treatment showed improvement or stabilization at 1 and 2 years of follow-up (Fig. 3C, Table S3, and Videos 1, 2, and 3).

Videos showing motor function before and after gene therapy are available at NEJM.org

DISCUSSION

Interim results for eight patients with MPSIH treated with HSPC gene therapy showed the feasibility of engineering hematopoiesis toward supraphysiologic IDUA enzyme secretion, which resulted in rapid and profound reduction of GAG storage, including in the CNS. The blood IDUA activity of treated children was 3 to 12 times as high as the mean of normal donors. Despite variable in vivo gene-marking levels in our cohort that may be overcome by future refinements in the transduction protocol, even the patients receiving HSPC gene therapy with engraftment of gene-corrected cells below 30% appeared to have higher blood IDUA activity than that which can be achieved by allogeneic hematopoietic stem-cell transplantation. An adequately powered case–control study is required to confirm differences in the depth of biochemical correction between HSPC gene therapy and allogeneic hematopoietic stem-cell transplantation. Nevertheless, residual accumulation of toxic metabolites in the tissues may cause organ dysfunction and may explain the considerable level of residual-disease burden after ERT and allogeneic hematopoietic stem-cell transplantation.[10,12,25-28] Longer follow-up is needed to understand whether and how a more complete metabolic correction of the biochemical MPSIH defect, as achieved by HSPC gene therapy, may improve clinical outcomes. In metachromatic leukodystrophy (MLD), enzyme overexpression by HSPC gene therapy was critically important for efficient tissue cross-correction, thus preventing or delaying progression of CNS and peripheral nervous system disease.[29,30] It is therefore plausible that incremental effects of HSPC gene therapy on neurologic outcome will be seen in MPSIH, as long as patients are treated before irreversible brain damage has occurred.

A known limitation of allogeneic hematopoietic stem-cell transplantation in patients with MPSIH is its failure to correct skeletal manifestations, which severely affect quality of life and activities of daily living.[10,31,32] Supraphysiologic IDUA enzyme levels may favor penetration into less vascularized skeletal tissues. Moreover, osteoclasts that are derived from genetically corrected myeloid precursors may release high levels of IDUA enzyme inside the bone microenvironment, mediating efficient cross-correction of neighboring osteocytes. Despite limited follow-up, our clinical data suggest that HSPC gene therapy may affect skeletal pathologic features, as evidenced by normal growth and stability or improvement of typical disease features on imaging, further supported by preclinical findings in the MPSIH disease model.[13] Longer observation is necessary to show the long-term effect of HSPC gene therapy on skeletal abnormalities in patients with MPSIH and to understand to what extent previously accumulated bone abnormalities may be reversible.

HSPC gene therapy may offer potential advantages over allogeneic hematopoietic stem-cell transplantation.[9,33,34] The use of autologous cells minimizes the risk of rejection and graft-versus-host disease, while allowing rapid reconstitution of innate and adaptive immunity, as reflected by the low incidence of post-transplantation complications in our HSPC gene therapy cohort, despite lympho- and myeloablative conditioning. The shortened transduction protocol including prostaglandin E_2 may have contributed to the rapid hematopoietic recovery and high polyclonality. We observed the disappearance of preexisting anti-IDUA antibodies in the five patients who were receiving ERT before treatment. Moreover, we did not find any evidence of immunogenicity against the transgene. Genotoxic effects from alkylating agents and insertional mutagenesis from the semirandomly integrating lentiviral vector[20] remain potential complications of HSPC gene therapy that merit careful monitoring, even though more than 10 years of follow-up from our MLD trials, in which the same lentiviral vector backbone has been used, did not highlight any event of clonal dominance.[30] Our finding that HSPC gene therapy results in metabolic cor-

rection in patients with MPSIH at engraftment levels below 30% may allow further reduction of conditioning-related toxic effects — for example, by considering antibody-based conditioning approaches in the future.

In this interim analysis, we found a favorable safety profile of HSPC gene therapy in patients with MPSIH along with encouraging metabolic and early clinical outcomes. These findings support further clinical development of this promising new therapy for MPSIH.

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APPENDIX

The authors' full names and academic degrees are as follows: Bernhard Gentner, M.D., Francesca Tucci, M.D., Stefania Galimberti, Ph.D., Francesca Fumagalli, M.D., Maurizio De Pellegrin, M.D., Paolo Silvani, M.D., Chiara Camesasca, M.D., Silvia Pontesilli, M.D., Silvia Darin, R.N., Francesca Ciotti, Psy.D., Marina Sarzana, P.T., Giulia Consiglieri, M.D., Chiara Filisetti, M.D., Giulia Forni, B.Sc., Laura Passerini, Ph.D., Daniela Tomasoni, B.Sc., Daniela Cesana, Ph.D., Andrea Calabria, Ph.D., Giulio Spinozzi, Ph.D., Maria-Pia Cicalese, M.D., Ph.D., Valeria Calbi, M.D., Maddalena Migliavacca, M.D., Ph.D., Federica Barzaghi, M.D., Ph.D., Francesca Ferrua, M.D., Ph.D., Vera Gallo, M.D., Simona Miglietta, M.Sc., Erika Zonari, Ph.D., Patali S. Cheruku, M.D., Claudia Forni, Ph.D., Marcella Facchini, Ph.D., Ambra Corti, B.Sc., Michela Gabaldo, Pharm.D., Stefano Zancan, M.Sc., Serena Gasperini, M.D., Attilio Rovelli, M.D., Jaap-Jan Boelens, M.D., Ph.D., Simon A. Jones, M.D., Robert Wynn, M.D., Cristina Baldoli, M.D., Eugenio Montini, Ph.D., Silvia Gregori, Ph.D., Fabio Ciceri, M.D., Maria G. Valsecchi, Ph.D., Giancarlo la Marca, Pharm.Sc., Rossella Parini, M.D., Luigi Naldini, M.D., Ph.D., Alessandro Aiuti, M.D., Ph.D., and Maria-Ester Bernardo, M.D., Ph.D.

The authors' affiliations are as follows: the Departments of Hematology and Bone Marrow Transplantation (B.G., F. Ciceri), Pediatric Immunohematology and Bone Marrow Transplantation (F.T., F. Fumagalli, S.D., F. Ciotti, M.S., G.C., C. Filisetti, M.-P.C., V.C., M.M., F.B., F. Ferrua, V.G., A.A., M.-E.B.), Neurology and Neurophysiology (F. Fumagalli), Pediatric Orthopedics (M.D.P.), Anesthesia and Critical Care (P.S.), Pediatric Cardiology (C.C.), and Neuroradiology (S.P., C.B.) and the San Raffaele Telethon Institute for Gene Therapy (B.G., F.T., F. Fumagalli, L.P., D.T., D.C., A.C., G.S., M.-P.C., V.C., M.M., F.B., F. Ferrua, V.G., S.M., E.Z., P.S.C., M.F., A.C., E.M., S. Gregori, R.P., L.N., A.A., M.-E.B.), IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University (F. Ciceri, L.N., A.A., M.-E.B.), and Bicocca Bioinformatics, Biostatistics, and Bioimaging Center, School of Medicine and Surgery, University of Milano–Bicocca (S. Galimberti, M.G.V.), Milan, Fondazione Telethon, Rome (C. Forni, M.G., S.Z.), the Department of Experimental and Clinical Biomedical Sciences, University of Florence, and Newborn Screening, Biochemistry and Pharmacology Laboratory, Meyer Children's University Hospital (G.F., G.M.), Florence, and the Department of Pediatrics, Fondazione Monza e Brianza per il Bambino e la sua Mamma, San Gerardo Hospital, Monza (S. Gasperini, A.R., R.P.) — all in Italy; Stem Cell Transplantation and Cellular Therapies, MSK Kids, Memorial Sloan Kettering Cancer Center, New York (J.-J.B.); and the Willink Unit, Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester University NHS Foundation Trust, University of Manchester (S.A.J.), and the Department of Blood and Marrow Transplantation, Royal Manchester Children's Hospital (R.W.) — both in Manchester, United Kingdom.

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