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# Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

J.R. Mendell, S. Al-Zaidy, R. Shell, W.D. Arnold, L.R. Rodino-Klapac, T.W. Prior, L. Lowes, L. Alfano, K. Berry, K. Church, J.T. Kissel, S. Nagendran, J. L'Italien, D.M. Sproule, C. Wells, J.A. Cardenas, M.D. Heitzer, A. Kaspar, S. Corcoran, L. Braun, S. Likhite, C. Miranda, K. Meyer, K.D. Foust, A.H.M. Burghes, and B.K. Kaspar

## ABSTRACT

### BACKGROUND

Spinal muscular atrophy type 1 (SMA1) is a progressive, monogenic motor neuron disease with an onset during infancy that results in failure to achieve motor milestones and in death or the need for mechanical ventilation by 2 years of age. We studied functional replacement of the mutated gene encoding survival motor neuron 1 (*SMN1*) in this disease.

### METHODS

Fifteen patients with SMA1 received a single dose of intravenous adeno-associated virus serotype 9 carrying SMN complementary DNA encoding the missing SMN protein. Three of the patients received a low dose ($6.7\times10^{13}$ vg per kilogram of body weight), and 12 received a high dose ($2.0\times10^{14}$ vg per kilogram). The primary outcome was safety. The secondary outcome was the time until death or the need for permanent ventilatory assistance. In exploratory analyses, we compared scores on the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale of motor function (ranging from 0 to 64, with higher scores indicating better function) in the two cohorts and motor milestones in the high-dose cohort with scores in studies of the natural history of the disease (historical cohorts).

### RESULTS

As of the data cutoff on August 7, 2017, all 15 patients were alive and event-free at 20 months of age, as compared with a rate of survival of 8% in a historical cohort. In the high-dose cohort, a rapid increase from baseline in the score on the CHOP INTEND scale followed gene delivery, with an increase of 9.8 points at 1 month and 15.4 points at 3 months, as compared with a decline in this score in a historical cohort. Of the 12 patients who had received the high dose, 11 sat unassisted, 9 rolled over, 11 fed orally and could speak, and 2 walked independently. Elevated serum aminotransferase levels occurred in 4 patients and were attenuated by prednisolone.

### CONCLUSIONS

In patients with SMA1, a single intravenous infusion of adeno-associated viral vector containing DNA coding for SMN resulted in longer survival, superior achievement of motor milestones, and better motor function than in historical cohorts. Further studies are necessary to confirm the safety and efficacy of this gene therapy. (Funded by AveXis and others; ClinicalTrials.gov number, NCT02122952.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Mendell at the Research Institute at Nationwide Children's Hospital, 700 Children's Dr., Columbus, OH 43205, or at jerry.mendell@nationwidechildrens.org.

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A Quick Take is available at NEJM.org

Spinal muscular atrophy (SMA) is a severe childhood monogenic disease resulting from loss or dysfunction of the gene encoding survival motor neuron 1 (*SMN1*). The incidence of this disease is approximately 1 in 10,000 live births, with a carrier frequency of 1 in 54.[1] SMA is characterized by the degeneration and loss of lower motor neurons, which leads to muscle atrophy. The disease is divided into four subtypes (1 through 4) on the basis of the age at onset and milestone achievement. SMA type 1 (SMA1) is the most severe form and most common genetic cause of death among infants.[2] There are two forms of *SMN*; *SMN1* is the primary gene responsible for functional production of SMN protein. *SMN2* preferentially excludes exon 7 during splicing[3] and, as a result, produces only a small fraction of functional SMN protein as compared with *SMN1*. Therefore, the *SMN2* copy number modifies the disease phenotype, and the presence of two copies of *SMN2* is associated with SMA1.[3] Infants with *SMN1* biallelic deletions and two copies of *SMN2* have a 97% risk of SMA1.

Recent studies of the natural history of SMA1 (historical cohort) showed that the median age at symptom onset among infants with the disease was 1.2 months (range, 0 to 4 months), and the disease was characterized by hypotonia, severe weakness from early infancy, and failure to sit without support.[4,5] In infants with SMA1 who have two copies of *SMN2*, the median age at death or the need for noninvasive ventilation for at least 16 hours per day for at least 14 consecutive days (considered equivalent to permanent ventilation) was 10.5 months.[4] In one cohort of affected children, only 25% survived without permanent ventilatory support at 13.6 months, and 8% survived without this support by 20 months.[4] Another prospective, multicenter historical study sponsored by the National Institutes of Health (NeuroNEXT)[5] involving patients with two copies of *SMN2* showed a median survival free of tracheostomy of 8 months (95% confidence interval, 6 to 17). All patients with SMA1 have a precipitous decline in respiratory and swallowing functions after birth and ultimately require mechanical nutritional support (through a nasogastric or gastrostomy tube) to maintain adequate nutrition and reduce the respiratory risks associated with aspiration. For patients with SMA1 in whom the onset of symptoms occurs by 3 months of age, most patients require feeding support by 12 months of age.[4]

Patients with SMA1 also do not achieve major milestones in motor function and have a decline in function, as measured on the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale, which ranges from 0 to 64, with higher scores indicating better motor function, a tool that is sensitive to minor changes in motor function, such as antigravity movements of limbs.[6-8] In a historical analysis of 34 patients with SMA1, all but 1 of the patients did not reach a score of at least 40 after 6 months of age.[4] In the NeuroNEXT cohort, CHOP INTEND scores decreased by a mean of 10.7 points from 6 months to 12 months of age.[5]

Therapeutic strategies to increase levels of SMN protein in motor neurons have focused on enhancing the effectiveness of *SMN2*. One approach has been central nervous system delivery of nusinersen (Ionis Pharmaceuticals/Biogen), an antisense oligonucleotide that was developed to inhibit exon 7 splicing in *SMN2*. This drug has been shown to improve weakness in the murine model of severe SMA and to increase the median life span of affected mice from 16 days to 25 days.[9,10] In December 2016, nusinersen was approved by the Food and Drug Administration for the treatment of SMA. This drug is administered by means of repeated intrathecal injections after four loading doses within the first 2 months of life.[11]

A potential alternative treatment for SMA1 is gene therapy, given as a one-time intravenous administration that delivers a copy of *SMN* in a self-complementary adeno-associated viral serotype 9 (scAAV9). (The coding region of this recombinant virus forms an intramolecular double-stranded DNA [or self-complementary] template.) This approach has induced SMN expression in motor neurons and peripheral tissues, which has countered the effects of SMA in a murine model and extended the average survival in this model from 15 days to 28.5 days with a low dose ($6.7\times10^{13}$ vg per kilogram of body weight) and to more than 250 days with higher doses of the vector ($2.0\times10^{14}$ and $3.3\times10^{14}$ vg per kilogram).[12-15]

In addition to crossing the blood–brain barrier and targeting central nervous system neurons at all regions of the spinal cord,[13] the systemic administration of AAV9-mediated gene therapy may be advantageous, given that SMN protein is ubiquitously expressed and SMA1 affects multiple systems (e.g., autonomic and enteric nervous systems, cardiovascular system, and pancreas[16,17]),

along with many cell types (e.g., heart,[18] pancreas,[16,17] and skeletal muscle[19]). The self-complementary feature of the vector combined with a hybrid cytomegalovirus enhancer–chicken beta-actin promoter enables rapid and sustained expression of *SMN*. In April 2014, we initiated a study of gene-replacement therapy involving infants with SMA1 who received a one-time dose of scAAV9 with delivery of the human survival motor neuron gene (h*SMN*), under control of the chicken beta-actin promoter (scAAV9.CB.h*SMN*) (AVXS-101).

## METHODS

### STUDY OVERSIGHT

The study was approved by the institutional review board at Nationwide Children's Hospital in Columbus, Ohio. Written informed consent was obtained from the parents or legal guardians of the children. The sponsor, AveXis, provided data management and statistical analysis; the gene and vector were provided by the vector-manufacturing facility at Nationwide Children's Hospital. All the authors vouch for the fidelity of the study conduct to the protocol (available with the full text of this article at NEJM.org) and for the accuracy and completeness of the data, analysis, and reporting of adverse events. An independent data and safety monitoring committee monitored the integrity and safety of the study.

### PATIENTS AND STUDY PROCEDURES

All the patients had a genetically confirmed diagnosis of SMA1, homozygous *SMN1* exon 7 deletions, and two copies of *SMN2*. Patients with the c.859G→C disease modifier in exon 7 of *SMN2* were excluded. Details regarding the inclusion and exclusion criteria are provided in the protocol.

Patients were enrolled in two cohorts, according to the dose of gene therapy that was administered. Patients in cohort 1 received a low dose ($6.7\times10^{13}$ vg per kilogram) and were enrolled from May 2014 through September 2014; those in cohort 2 received a high dose ($2.0\times10^{14}$ vg per kilogram) and were enrolled from December 2014 through December 2015. As a result of serum aminotransferase elevations in Patient 1 in cohort 1, which led to a protocol amendment, Patients 2 through 15 received oral prednisolone at a dose of 1 mg per kilogram per day for approximately 30 days, starting 24 hours before the administration of gene vector. (Details regarding this amendment are provided in the Supplementary Appendix, available at NEJM.org.)

The vector was delivered in normal saline (approximately 10 to 20 ml per kilogram) that was infused intravenously during a period of approximately 60 minutes. At the time of enrollment, some patients required enteral feeding by means of a gastrostomy or nasogastric tube, the choice of which was based on the preference of the parents or the primary physician. Once enrolled in the study, all the patients who required nutritional support underwent placement of a gastrostomy tube, and the tubes were not removed during the study.[20]

### OUTCOMES

The primary outcome was the determination of safety on the basis of any treatment-related adverse events of grade 3 or higher. The secondary outcome was the time until death or the need for permanent ventilatory assistance. The latter was defined as at least 16 hours of respiratory assistance per day continuously for at least 14 days in the absence of an acute, reversible illness or a perioperative state. Exploratory outcomes included motor-milestone achievements (particularly, sitting unassisted) and CHOP INTEND scores.[7,8]

The maintenance of scores of more than 40 points has been considered to be clinically meaningful in SMA in the application of the CHOP INTEND scale.[4,5] Sitting unassisted was evaluated and classified according to the following criteria: sitting unassisted for at least 5 seconds, according to item 22 of the Bayley Scales of Infant and Toddler Development gross motor subtest ("sitting unassisted")[21]; sitting unassisted for at least 10 seconds, according to the World Health Organization (WHO) criteria ("sitting unassisted per WHO criteria")[22]; and sitting unassisted for at least 30 seconds, according to item 26 of the Bayley Scales mentioned above ("independent functional sitting"). Major motor milestones were confirmed by means of an examination of video recordings of the patients by an independent reviewer. Compound muscle action potentials were recorded from surface electrodes at baseline and every 6 months after infusion. Data regarding compound muscle action potentials have not been fully analyzed and are not reported here.

### STATISTICAL ANALYSIS

Safety analyses were performed in all the patients, who were also included in the primary

**Table 1. Demographic and Clinical Characteristics of the 15 Patients.***

| Characteristic | Cohort 1 (N=3) | Cohort 2 (N=12) |
|---|---|---|
| Mean age (range) — mo | 6.3 (5.9–7.2) | 3.4 (0.9–7.9) |
| Mean weight (range) — kg | 6.6 (6.0–7.1) | 5.7 (3.6–8.4) |
| Sex — no. (%) | | |
| Male | 1 (33) | 5 (42) |
| Female | 2 (67) | 7 (58) |
| Race — no. (%)† | | |
| White | 3 (100) | 11 (92) |
| Other | 0 | 1 (8) |
| Mean age at symptom onset (range) — mo | 1.7 (1.0–3.0) | 1.4 (0–3.0) |
| Mean age at genetic diagnosis (range) — days‡ | 33 (4–85) | 60 (0–136) |
| Mean score on CHOP INTEND scale (range)§ | 16 (6–27) | 28 (12–50) |
| Patients with clinical support — no. (%) | | |
| Nutritional | 3 (100) | 5 (42) |
| Ventilatory | 3 (100) | 2 (17) |

* Of the 15 study patients, the 3 patients in cohort 1 received a low dose of adeno-associated virus serotype 9 carrying *SMN* ($6.7\times10^{13}$ vg per kilogram) and the 12 patients in cohort 2 received a high dose ($2.0\times10^{14}$ vg per kilogram).
† Race was reported by the parents of the patients.
‡ In one patient in cohort 2, the diagnosis was made prenatally, so an age of 0 was reported at the time of genetic diagnosis.
§ Scores on the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale of motor function range from 0 to 64, with higher scores indicating better function.

analysis of survival (as defined above and in the protocol) and in analyses of changes on the CHOP INTEND scale from baseline to 1 month and 3 months. Such changes from baseline to each study visit were analyzed with the use of a mixed-effects model for repeated measurements. The mixed model included the fixed effects of cohort and visit and a covariate of baseline score. Milestone achievements and nutritional and ventilatory support were analyzed in cohort 2. Statistical analyses were performed with the use of SAS software, version 9.4. All comparisons with historical cohorts were solely descriptive.

## RESULTS

### PATIENTS

Of the 16 patients who were screened, 1 was excluded because of persistently elevated anti-AAV9 antibody titers (>1:50). Of the 15 patients who were included in the study, 3 were enrolled in the low-dose cohort 1 and 12 were enrolled in the high-dose cohort 2. The mean age of patients at the time of treatment was 6.3 months (range, 5.9 to 7.2) in cohort 1 and 3.4 months (range, 0.9 to 7.9) in cohort 2 (Table 1).

### SURVIVAL AND PERMANENT VENTILATION

As of August 7, 2017, all the patients had reached an age of at least 20 months and did not require permanent mechanical ventilation; the median age at their last pulmonary assessment was 30.8 months in cohort 1 and 25.7 months in cohort 2 (Fig. 1). In contrast, only 8% of the patients in a historical cohort did not require permanent mechanical ventilation.[4] At 29 months of age, one patient in cohort 1 required permanent ventilation because of hypersalivation. After salivary-gland ligation, the requirement for the use of noninvasive ventilation was reduced by 25% to 15 hours per day.

### MOTOR FUNCTION ASSESSMENTS

All the patients in cohorts 1 and 2 had increased scores from baseline on the CHOP INTEND scale and maintained these changes during the study (Fig. 2). Patients in cohort 2 had mean increases of 9.8 points at 1 month and 15.4 points at 3 months ($P<0.001$ for both comparisons); 11 patients attained and sustained scores of more than 40 points. At the study cutoff on August 7, 2017, patients in cohort 1 had a mean increase of 7.7 points from a mean baseline of 16.3 points, and those in cohort 2 had a mean increase of 24.6 points from a mean baseline of 28.2 points.

### MOTOR MILESTONES IN COHORT 2

A total of 11 of 12 patients in cohort 2 were able to sit unassisted for at least 5 seconds, 10 for at least 10 seconds, and 9 for at least 30 seconds (Table 2). A total of 11 achieved head control, 9 could roll over, and 2 were able to crawl, pull to stand, stand independently, and walk independently. Eleven patients attained the ability to speak. No patients in the historical cohorts had achieved any of these motor milestones and rarely had achieved the ability to speak.[6,23]

### PULMONARY AND NUTRITIONAL STATUS IN COHORT 2

Among the 12 patients in cohort 2, 10 did not require noninvasive ventilation at baseline as compared with 7 who were independent of ventilatory

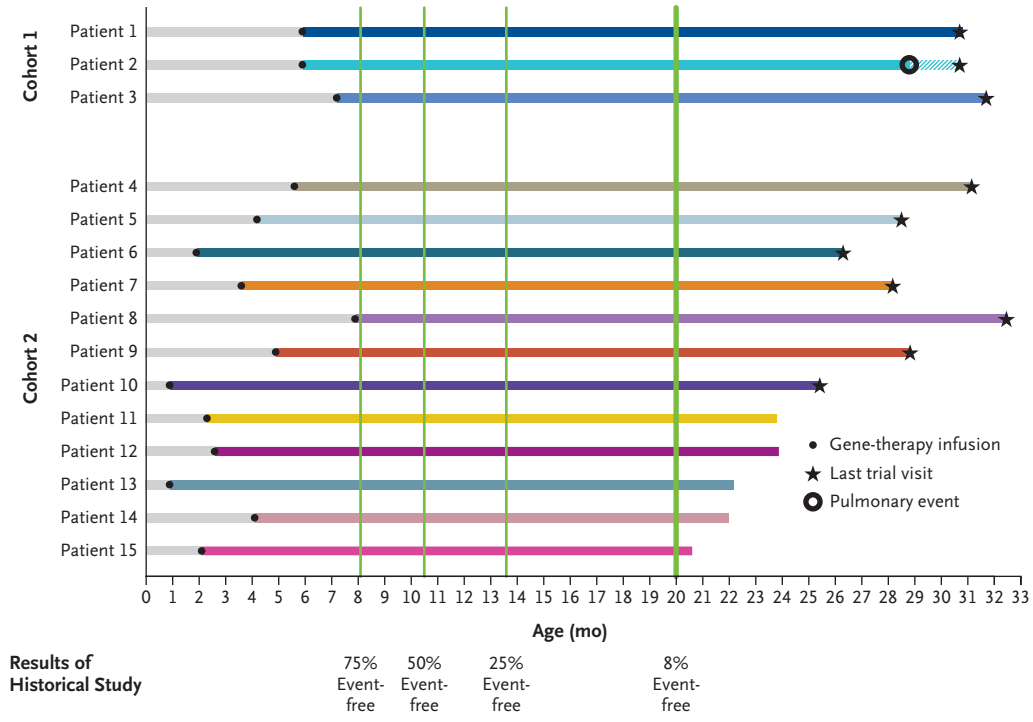


**Figure 1. Survival Free from Permanent Ventilation in the 15 Study Patients.**

Shown is the duration of survival free from the need for permanent ventilation for the 3 patients in cohort 1, who received a low dose of adeno-associated viral vector containing DNA coding for SMN ($6.7\times10^{13}$ vg per kilogram), and the 12 patients in cohort 2, who received a high dose ($2.0\times10^{14}$ vg per kilogram). Stars indicate the completion of the ongoing 2-year safety follow-up. The percentages of patients who were event-free in a historical study of spinal muscular atrophy conducted by the Pediatric Neuromuscular Clinical Research Network[4] are provided at the bottom of the graph for a control comparison, as indicated by the vertical green lines. The thicker vertical line indicates the benchmark of 20 months, at which time only 8% of the patients with this disease typically survive without permanent ventilation.

assistance at the last follow-up visit (Table 2). At baseline, 7 patients did not require enteral feeding, including 1 who later required placement of a gastrostomy tube after gene-replacement therapy, possibly in association with scoliosis surgery. Of the 5 patients who had received enteral feeding before gene-replacement therapy, at the last follow-up, 11 of the 12 patients had achieved or retained the ability to swallow independently and 4 were able to feed orally.

### SAFETY

As of August 7, 2017, a total of 56 serious adverse events were observed in 13 patients in the two cohorts. Of these events, investigators determined that 2 events were treatment-related grade 4 events on the basis of laboratory values, according to Common Terminology Criteria for Adverse Events (Table 3). Patient 1 in cohort 1 had elevations in serum aminotransferase levels (31 times the upper limit of the normal range for alanine aminotransferase [ALT] and 14 times the upper limit for aspartate aminotransferase [AST]) without other liver-function abnormalities (i.e., total and indirect bilirubin and alkaline phosphatase) and without clinical manifestations. As described above, these elevations were attenuated by prednisolone treatment, which was subsequently administered in the remaining patients. One patient in cohort 2 required additional prednisolone to attenuate elevated serum ALT and AST levels (35 times the upper limit of the normal range for ALT and 37 times for AST). Of the 241 nonserious adverse events, 3 were deemed to be treatment-related and consisted of asymptomatic elevations in serum aminotransferase levels in 2 patients (ALT and AST, both less than 10 times the upper limit of the normal range), which

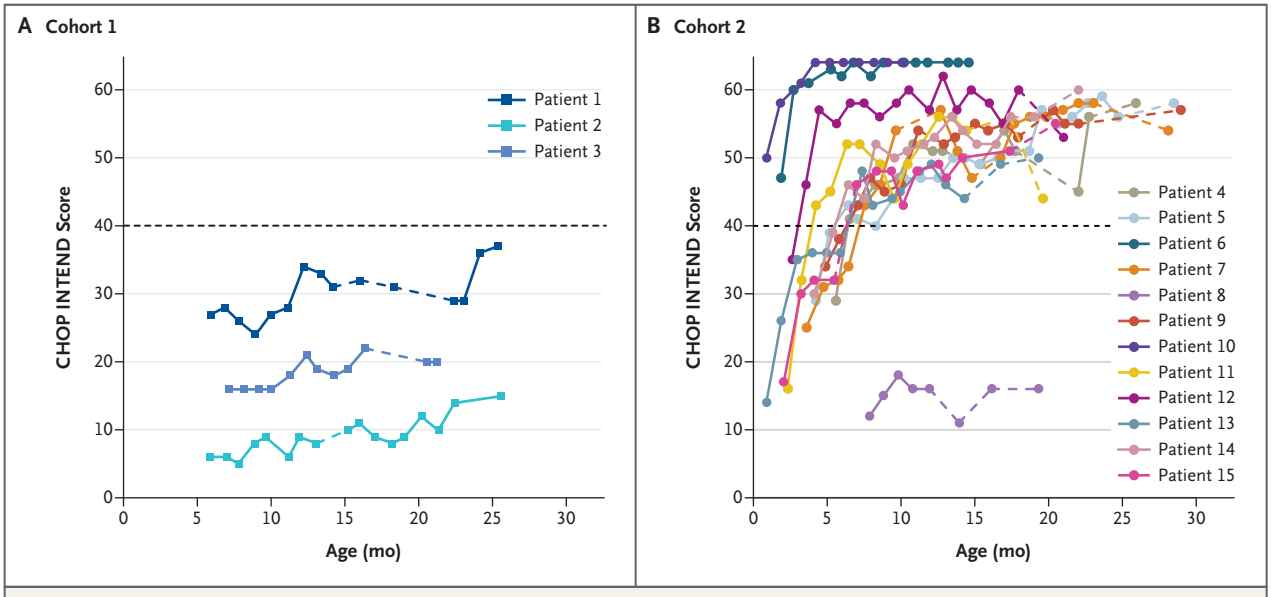


**Figure 2. Motor Function after Gene Therapy.**

Shown are changes in the score for motor function on the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale among the 3 patients in cohort 1 (Panel A) and the 12 patients in cohort 2 (Panel B) who received gene therapy with adeno-associated viral vector containing DNA coding for SMN. The scale ranges from 0 to 64, with higher scores indicating better motor function; historical controls with spinal muscular atrophy type 1 never reach 40 points (indicated by the black dashed line). The dashed lines on the individual patient curves indicate either a missed assessment or a partial assessment because of illness, lack of cooperation, or fatigue of the patient; such data were not included in the analyses. The timing of the administration of gene therapy in Figure 1 can be matched with the data shown here for each patient.

were resolved without additional prednisolone treatment (Table 3). There were no other abnormalities on liver-function testing. Of the 15 patients, 14 had respiratory illnesses, which in children with SMA1 frequently result in death or the need for tracheostomy.

## DISCUSSION

A single intravenous infusion of adeno-associated viral vector containing DNA coding for SMN in patients with SMA1 resulted in longer survival than in historical cohorts with this disease.[4] All 15 patients surpassed the previously reported median age of survival without permanent ventilation of 10.5 months[4] for patients with SMA1 with two *SMN2* copies. All the patients also surpassed the benchmark of 20 months, at which time only 8% of the patients with this disease typically survive without permanent ventilation.[4] Of the 12 patients in cohort 2, all but 1 achieved motor-function milestones that have not been reported in historical cohorts.[4,6] The attained motor function was clinically meaningful, as reflected by feeding (hand to mouth), sitting, and talking. The majority of the patients who did not require supportive care at enrollment were free of nutritional support (6 of 7 patients) and ventilatory support (7 of 10 patients) at the last follow-up visit.[4,24] In the two cohorts, the patients had increases in the score on the CHOP INTEND scale from baseline. Within the first month in cohort 2, the mean increase was 9.8 points, in contrast to a decline of a mean of more than 10 points between 6 and 12 months of age in the historical cohort in the NeuroNEXT study.[5]

Preclinical studies of *SMN* gene-replacement therapy in the SMNΔ7 mouse model showed improvements in survival and motor function with early treatment, presumably at a time when motor neurons are still intact.[14] The clinical findings in our study of early treatment reflected the direction of those in the preclinical studies. Two patients were able to crawl, stand, and walk without support after early treatment. Both of these patients had a family history of SMA, which probably contributed to the early diagno-

**Table 2. Event-free Survival and Motor and Other Milestones among the 12 Patients in Cohort 2.***

| Variable | Age at Study Entry | Event-free Survival† | Motor Milestones | | | | | | | Other Achievements | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brings Hand to Mouth | Controls Head | Rolls Over‡ | Sits with Assistance | Sits Unassisted§ | | | Speaks | Swallows | No NIV Use | No Nutritional Support¶ |
| | *mo* | | | | | | *≥5 sec* | *≥10 sec* | *≥30 sec* | | | | |
| **Patient no.** | | | | | | | | | | | | | |
| 4 | 5.6 | 31.1 | + | + | + | + | + | | | + | + | | |
| 5 | 4.2 | 28.5 | + | + | + | + | + | + | + | + | + | + | + |
| 6 | 1.9 | 26.1 | + | + | + | + | + | + | + | + | + | + | + |
| 7 | 3.6 | 28.1 | + | + | + | + | + | + | | + | + | + | |
| 8 | 7.9 | 32.4 | + | | | | | | | | | | |
| 9 | 4.9 | 28.9 | + | + | + | + | + | + | + | + | + | + | + |
| 10 | 0.9 | 25.3 | + | + | + | + | + | + | + | + | + | + | + |
| 11 | 2.3 | 23.8 | + | + | + | + | + | + | + | + | + | | |
| 12 | 2.6 | 23.9 | + | + | + | + | + | + | + | + | + | + | + |
| 13 | 0.9 | 22.1 | + | + | | + | + | + | + | + | + | | |
| 14 | 4.1 | 22.0 | + | + | + | + | + | + | + | + | + | + | + |
| 15 | 2.1 | 20.6 | + | + | | + | + | + | + | + | + | | |
| **Patients with outcome (%)** | | | | | | | | | | | | | |
| This study | | 100 | 100 | 92 | 75 | 92 | 92 | 83 | 75 | 92 | 92 | 58 | 50 |
| Natural-history studies | | 8 by 20 mo‖ | NA | 0 | 0** | 0** | 0** | 0** | 0** | NA | NA | NA | 8 by 20 mo‖ |

* At baseline, none of the patients in cohort 2 had achieved any of the listed motor milestones except for bringing a hand to the mouth. As of August 7, 2017, the majority of these patients had reached at least one major motor milestone. No patients in cohort 1 are listed, since none attained any motor milestones. NA denotes not available, and NIV noninvasive ventilation. Plus signs indicate achievement of milestone.

† Event-free survival (the primary efficacy outcome) was defined as the age at the last follow-up at which patients were free of ventilatory support, which was defined as the need for ventilation for at least 16 hours per day for at least 14 consecutive days.

‡ According to item 20 on the Bayley Scales of Infant and Toddler Development, rolling over is defined as movement of at least 180 degrees both left and right from a position of lying on the back.

§ Sitting unassisted for at least 5 seconds is in accordance with the criteria of item 22 on the Bayley Scales of Infant and Toddler Development gross motor subtest and surpasses the 3-second count that is used as a basis for sitting (test item 1) on the Hammersmith Functional Motor Scale–Expanded for Spinal Muscular Atrophy (SMA). Sitting unassisted for at least 10 seconds is in accordance with the criteria used in the World Health Organization Multicentre Growth Reference Study. Sitting unassisted for at least 30 seconds defines functional independent sitting and is in accordance with the criteria of item 26 on the Bayley Scales of Infant and Toddler Development gross motor subtest.

¶ Nutritional support refers to the placement of either a gastrostomy tube or a nasogastric tube, as determined by the preference of the parents or the primary physician. Once enrolled in the study, all the patients who required nutritional support underwent gastrostomy-tube placement, and none were removed during the study.

‖ Data are from Finkel et al.[4]

** Data are from De Sanctis et al.[6]

**Table 3. Adverse Events.***

| Event | Cohort 1 (N=3) | | Cohort 2 (N=12) | | All Patients (N=15) | |
|---|---|---|---|---|---|---|
| | Events | Patients | Events | Patients | Events | Patients |
| | *no.* | *no. (%)* | *no.* | *no. (%)* | *no.* | *no. (%)* |
| Any adverse event | 44 | 3 (100) | 253 | 12 (100) | 297 | 15 (100) |
| Any serious adverse event | 7 | 3 (100) | 49 | 10 (83) | 56 | 13 (87) |
| Adverse event associated with treatment† | 1 | 1 (33) | 4 | 3 (25) | 5 | 4 (27) |
| Common adverse event | | | | | | |
| Upper respiratory tract infection | 3 | 1 (33) | 26 | 10 (83) | 29 | 11 (73) |
| Vomiting | 0 | 0 | 11 | 8 (67) | 11 | 8 (53) |
| Constipation | 1 | 1 (33) | 9 | 7 (58) | 10 | 8 (53) |
| Pyrexia | 1 | 1 (33) | 10 | 6 (50) | 11 | 7 (47) |
| Nasal congestion | 0 | 0 | 8 | 6 (50) | 8 | 6 (40) |
| Gastroesophageal reflux | 1 | 1 (33) | 6 | 5 (42) | 7 | 6 (40) |
| Enterovirus infection | 1 | 1 (33) | 7 | 4 (33) | 8 | 5 (33) |
| Pneumonia | 0 | 0 | 11 | 5 (42) | 11 | 5 (33) |
| Rhinovirus infection | 1 | 1 (33) | 10 | 4 (33) | 11 | 5 (33) |
| Cough | 0 | 0 | 9 | 5 (42) | 9 | 5 (33) |
| Otitis media | 6 | 2 (67) | 3 | 2 (17) | 9 | 4 (27) |
| Elevated aminotransferase level | 1 | 1 (33) | 3 | 3 (25) | 4 | 4 (27) |
| Respiratory failure | 1 | 1 (33) | 5 | 3 (25) | 6 | 4 (27) |
| Parainfluenza virus infection | 1 | 1 (33) | 4 | 3 (25) | 5 | 4 (27) |
| Rash | 0 | 0 | 5 | 4 (33) | 5 | 4 (27) |
| Atelectasis | 0 | 0 | 4 | 4 (33) | 4 | 4 (27) |
| Viral gastroenteritis | 0 | 0 | 4 | 4 (33) | 4 | 4 (27) |
| Rhinorrhea | 0 | 0 | 4 | 3 (25) | 4 | 3 (20) |
| Bronchiolitis | 0 | 0 | 3 | 3 (25) | 3 | 3 (20) |
| Diarrhea | 0 | 0 | 3 | 3 (25) | 3 | 3 (20) |
| Ear infection | 1 | 1 (33) | 2 | 2 (17) | 3 | 3 (20) |
| Injury from fall | 0 | 0 | 3 | 3 (25) | 3 | 3 (20) |
| Human rhinovirus | 0 | 0 | 3 | 3 (25) | 3 | 3 (20) |
| Streptococcal pharyngitis | 1 | 1 (33) | 2 | 2 (17) | 3 | 3 (20) |
| Respiratory syncytial virus | | | | | | |
| Pneumonia | 1 | 1 (33) | 2 | 2 (17) | 3 | 3 (20) |
| Bronchiolitis | 1 | 1 (33) | 2 | 2 (17) | 3 | 3 (20) |
| Viral upper respiratory tract infection | 0 | 0 | 3 | 3 (25) | 3 | 3 (20) |

* Listed are adverse events that had been reported in at least 15% of the patients by the data cutoff on August 7, 2017.
† Included in this category are all the adverse events (including elevations in aminotransferase levels) that were definitely related to gene therapy, according to investigator assessment. Details regarding the elevated aminotransferase levels and their treatment are provided in the Safety subsection of the Results section.

sis. Although all the patients in the two cohorts in our study have continued to have improvements in motor function, the preclinical and clinical data suggest a benefit for early treatment and newborn screening for SMA.[6]

Serious adverse events caused by AAV gene-replacement therapy were limited to elevated serum aminotransferase levels without other liver enzyme abnormalities approximately 3 weeks after treatment in two patients; two other patients had elevations that did not reach the cutoff for the definition of serious adverse events (i.e., >10 times the normal range). Elevations in liver enzymes were attenuated by prednisolone treatment. One patient did not pass screening owing to the presence of anti-AAV9 antibody, which is consistent with population studies that suggest a low rate of anti-AAV9 seropositivity among children and young adults and increasing rates of anti-AAV9 seropositivity among persons older than 40 years of age.[25] However, the presence of antibodies to the virus may be a limitation of AAV gene-replacement therapy.

This study used a single-group design with a historical cohort as a control, which is one of a limited number of options when the natural history of a disease is well characterized and lethal. In order to enroll a homogeneous sample that was similar to those in published historical studies, we restricted enrollment to include only symptomatic patients with SMA1 who had biallelic *SMN1* mutations and two *SMN2* copies and did not enroll patients with the c.859G→C genetic modifier in exon 7 of *SMN2*,[26] since this genetic modifier predicts a milder phenotype of the disease.

In conclusion, a one-time intravenous infusion of a high dose of adeno-associated viral vector containing DNA coding for SMN in patients with SMA1 resulted in extended survival, improved motor function, and increased scores on the CHOP INTEND scale to levels that had not previously been observed in this disease. Such improvements resulted in a lower percentage of patients who needed supportive care than those in historical studies. At the time of this report, no waning of effect or clinical regression in motor function had been reported in follow-up of up to 2 years. Several patients had transient and asymptomatic elevations in aminotransferase levels. Further studies are necessary to assess the long-term safety and durability of gene-replacement therapy in patients with SMA1.

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## APPENDIX

The authors' full names and academic degrees are as follows: Jerry R. Mendell, M.D., Samiah Al-Zaidy, M.D., Richard Shell, M.D., W. Dave Arnold, M.D., Louise R. Rodino-Klapac, Ph.D., Thomas W. Prior, Ph.D., Linda Lowes, P.T., Ph.D., Lindsay Alfano, D.P.T., Katherine Berry, P.T., Kathleen Church, M.S.W., John T. Kissel, M.D., Sukumar Nagendran, M.D., James L'Italien, Ph.D., Douglas M. Sproule, M.D., Courtney Wells, B.S., Jessica A. Cardenas, Ph.D., Marjet D. Heitzer, Ph.D., Allan Kaspar, Ph.D., Sarah Corcoran, B.S., Lyndsey Braun, B.S., Shibi Likhite, Ph.D., Carlos Miranda, Ph.D., Kathrin Meyer, Ph.D., K.D. Foust, Ph.D., Arthur H.M. Burghes, Ph.D., and Brian K. Kaspar, Ph.D.

The authors' affiliations are as follows: the Center for Gene Therapy at the Research Institute at Nationwide Children's Hospital (J.R.M., S.A.-Z., L.R.R.-K., L.L., L.A., K.B., K.C., S.L., C.M., K.M., B.K.K.) and the Departments of Pediatrics (J.R.M., S.A.-Z., R.S., L.L., L.A., K.B., K.C., J.T.K., B.K.K.), Neurology (J.R.M., W.D.A., L.R.R.-K., A.H.M.B., B.K.K.), Pathology (T.W.P.), and Molecular and Cellular Biochemistry (A.H.M.B.), Ohio State University — both in Columbus; and AveXis, Bannockburn, IL (S.N., J.L., D.M.S., C.W., J.A.C., M.D.H., A.K., S.C., L.B., K.D.F., B.K.K.).

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