BRIEF REPORT

SOD1 Suppression with Adeno-Associated Virus and MicroRNA in Familial ALS

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SUMMARY

Two patients with familial amyotrophic lateral sclerosis (ALS) and mutations in the gene encoding superoxide dismutase 1 (*SOD1*) were treated with a single intrathecal infusion of adeno-associated virus encoding a microRNA targeting SOD1. In Patient 1, SOD1 levels in spinal cord tissue as analyzed on autopsy were lower than corresponding levels in untreated patients with SOD1-mediated ALS and in healthy controls. Levels of SOD1 in cerebrospinal fluid were transiently and only slightly lower in Patient 1 but were not affected in Patient 2. In Patient 1, meningoradiculitis developed after the infusion; Patient 2 was pretreated with immunosuppressive drugs and did not have this complication. Patient 1 had transient improvement in the strength of his right leg, a measure that had been relatively stable throughout his disease course, but there was no change in his vital capacity. Patient 2 had stable scores on a composite measure of ALS function and a stable vital capacity during a 12-month period. This study showed that intrathecal microRNA can be used as a potential treatment for SOD1-mediated ALS.

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In 10% of patients with amyotrophic lateral sclerosis (ALS), the disease is hereditary and is caused by single-gene mutations.[1] In animal models of familial ALS resulting from mutations in the gene encoding superoxide dismutase 1 (*SOD1*), suppression of the mutated gene increases survival.[2-5] In monkeys, an intrathecal infusion of adeno-associated virus rh10 containing an anti-SOD1 microRNA (AAV-miR-SOD1) has been shown to degrade *SOD1* messenger RNA, thereby repressing the expression of the gene in the spinal cord.[6,7] We tested the safety of infusion of AAV-miR-SOD1 in two patients with ALS caused by *SOD1* mutations. Measures of clinical efficacy were exploratory.

METHODS

Clinical-grade AAV-miR-SOD1 was produced at Nationwide Children's Hospital (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The clinical protocol (also available at NEJM.org) was authorized by the Western Institutional Review Board and the Food and Drug Administration. The RNA sequence is described in the Supplementary Appendix. The dose of virus that was administered was extrapolated from studies in animals.[6,7] The primary outcome was safety.

The clinical course of the two patients was prospectively obtained, as described in the protocol. The measures that were used were the ALS Functional Rating Scale–Revised (ALSFRS-R; a standardized 12-item questionnaire regarding functional status, with scores ranging from 0 to 48 points and with higher scores indicating better function)[8]; the slow vital capacity, as measured on the EasyOne Plus Spirometer (NDD Medical Technologies); the dynamometric measurement of 12 muscles[9-11] (normalized with respect to age, sex, and weight) with the Accurate Test of Limb Isometric Strength (on a scale that ranges from 0 to 150% of normal); and the Medical Research Council (MRC) grading of strength on a scale from 0 (no movement) to 5 (normal strength in a muscle group).

Patient 1 was treated at the University of Massachusetts Medical School and was assessed monthly by two neurologists (who performed MRC testing) and two project coordinators (who measured functional status and limb strength). Patient 2 was treated at Massachusetts General Hospital and was assessed by a neurologist (who performed MRC testing), a clinical research coordinator (who evaluated functional status), and two physical therapists (who evaluated limb strength). To date, these are the only two patients we have treated with this microRNA method in our investigator-initiated study, which had no industry involvement.

PATIENT 1

During the month of February 2017, Patient 1, a 22-year-old man, began to notice weakness in his left leg. He had the same *SOD1* missense mutation replacing alanine with valine at position 5 (*SOD1*-A5V) as his mother, who had died from ALS at the age of 45 years. In March 2017, his slow vital capacity was 100% of the predicted value, and his ALSFRS-R score was 42 (Fig. 1A and 1B). The flexion strength in his left hip was MRC grade 3, which indicated that he could move the limb against gravity. He could not bear full weight on his left heel or toes.

On July 19, 2017, he received a single intrathecal infusion of 4.2×10^{14} vector genomes of AAV-miR-SOD1 along with an intravenous bolus of methylprednisolone (1.0 g); the latter was repeated the following day. Oral prednisone (at a dose of 60 mg per day) was then initiated, with planned tapering during a 4-week period. At that time, there was no plantar flexion or dorsiflexion in the left ankle (MRC grade, 0); the left knee flexion and extension were both MRC grade 2, indicating movement only with gravity eliminated; the left hip flexion and extension were MRC grade 0 and grade 4, respectively. The strength of the right leg and both arms was normal, as were sensory function and cognition. Dynamometry showed that dorsiflexion strength in the left ankle was not measurable; extension and flexion in the left knee were at 3% and 11% of the predicted strength, respectively (Fig. 2), and elbow flexion and extension strength on the left side were approximately 25% of the predicted value. Deep tendon reflexes were normal, and Babinski signs were absent. In the cerebrospinal fluid (CSF), the protein level was 74 mg per deciliter (reference value, 15 to 45), and there were 3 white cells per cubic millimeter (Table S1).

Three weeks after the infusion, he had transient tingling in both hands, and 1 week later, he reported having a feeling of painful "electric shocks" in his left foot. The prednisone dose, which had been tapered to 10 mg per day, was increased to 30 mg. At 8 weeks, the CSF had 23 white cells per cubic millimeter and a protein level of 342 mg per deciliter (Table S1). Blood levels of hepatic aminotransferases had increased, with the alanine aminotransferase level peaking at 659 U per liter (upper limit of the normal range, 40 U per liter) at 10 weeks (Fig. S2A). At 10 weeks, sural sensory-nerve action potentials, left median sensory potentials, and H-reflexes (or Hoffmann's reflexes), which were normal before treatment, were absent. The amplitudes of superficial peroneal-nerve sensory potentials were reduced from 18 μV at the time he entered the study to 6 μV on the left side and from 24 to 5 μV on the right side. Left peroneal motor-conduction velocities on the left side were reduced from 64.9 m per second at baseline to 35.0 m per second. Evoked potentials in the median nerve were delayed on the left side (0.6 msec to Erb's point). At 16 weeks, magnetic resonance imaging of the lumbar region showed contrast enhance-

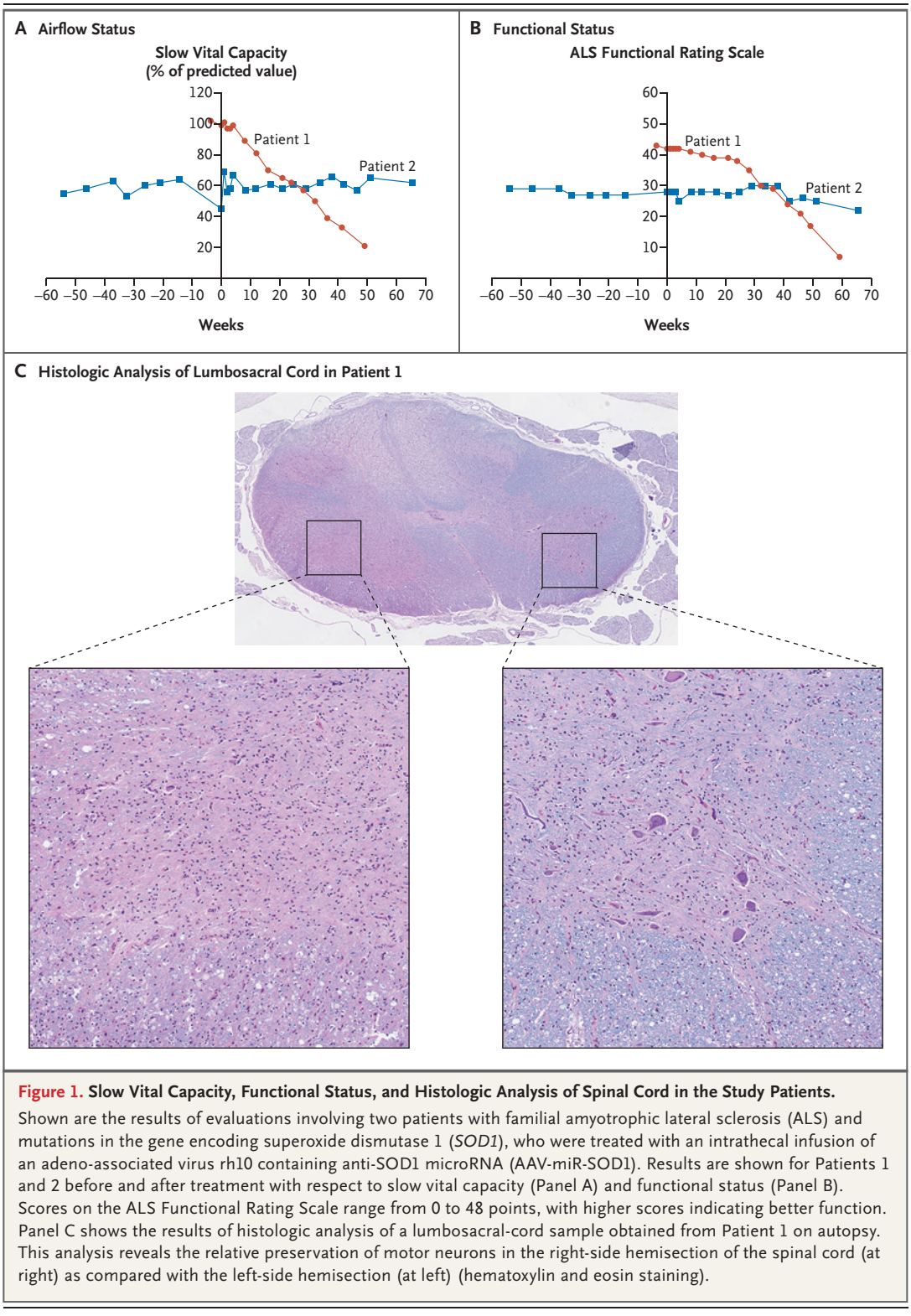

Figure 1. Slow Vital Capacity, Functional Status, and Histologic Analysis of Spinal Cord in the Study Patients.

Shown are the results of evaluations involving two patients with familial amyotrophic lateral sclerosis (ALS) and mutations in the gene encoding superoxide dismutase 1 (*SOD1*), who were treated with an intrathecal infusion of an adeno-associated virus rh10 containing anti-SOD1 microRNA (AAV-miR-SOD1). Results are shown for Patients 1 and 2 before and after treatment with respect to slow vital capacity (Panel A) and functional status (Panel B). Scores on the ALS Functional Rating Scale range from 0 to 48 points, with higher scores indicating better function. Panel C shows the results of histologic analysis of a lumbosacral-cord sample obtained from Patient 1 on autopsy. This analysis reveals the relative preservation of motor neurons in the right-side hemisection of the spinal cord (at right) as compared with the left-side hemisection (at left) (hematoxylin and eosin staining).

ment in the cauda equina and some dorsal root ganglia (Fig. S3). Over a period of several weeks, the pain in the left foot decreased but did not resolve.

Twenty-four weeks after treatment (46 weeks after the onset of ALS symptoms), the patient's ALSFRS-R score was reduced to 38 from the baseline level of 42. The loss of strength in the left leg continued, with left hip flexion and extension of MRC grade 0 and grade 2, respectively. Scores for strength of extension and flexion of the left knee were absent, whereas scores for extension and flexion of the right knee were 85% and 61%, respectively, with both scores better than at 20 weeks. At 36 weeks after treatment, flexion of the right knee was 100% of the predicted value on dynamometry. At 41 weeks, the extension and flexion of the right knee were 90% and 56%, respectively, of the predicted values (Fig. 2). The CSF showed 8 white cells per

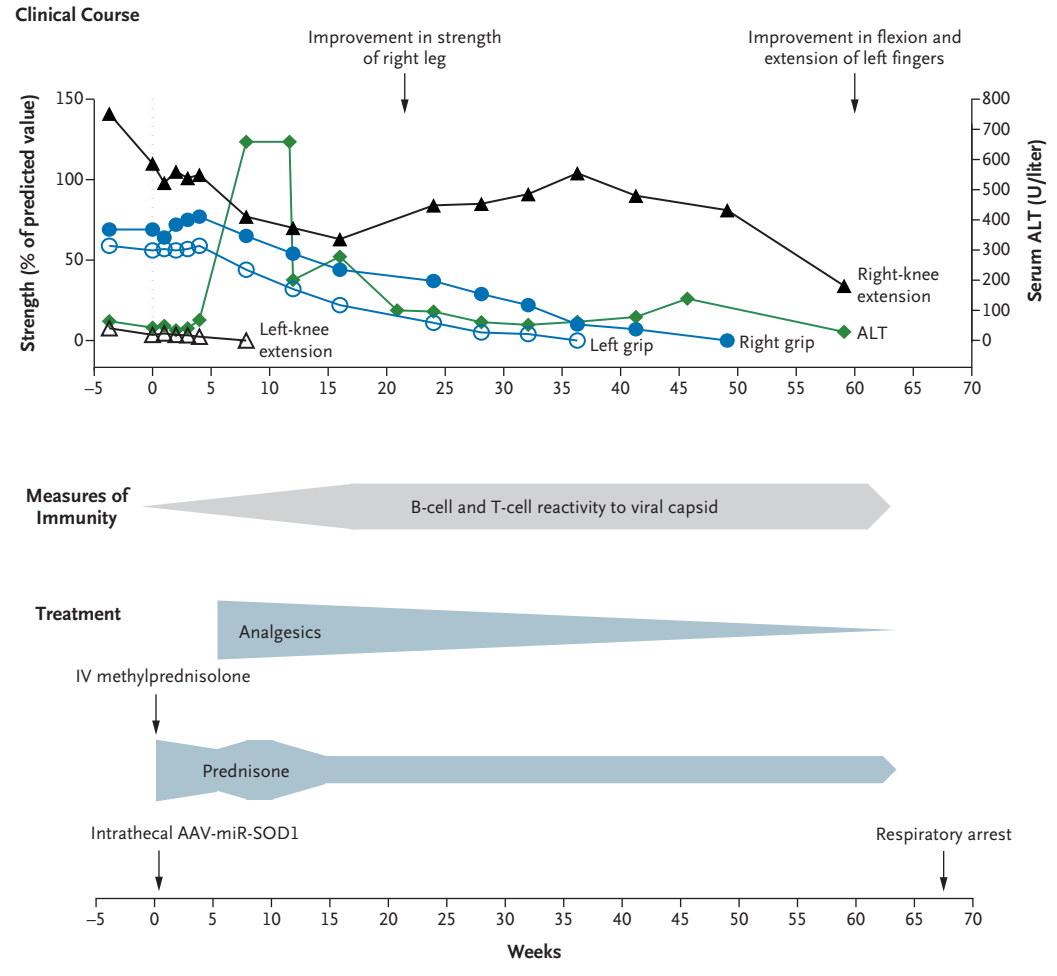

Figure 2. Time Course of Clinical Events, Immune Reaction, and Treatment in Patient 1.

The top portion of the graph shows the time course of dynamometric measurement on the Accurate Test of Limb Isometric Strength with respect to the patient's grip on the right side (blue solid circles) and left side (blue open circles), as well as knee extension on the right side (black solid triangles) and left side (black open triangles). The dynamometric force is presented as a fraction of the predicted value as compared with normalized data for age- and sex-matched controls. The time course of the increase in the alanine aminotransferase (ALT) level is also shown (green diamonds), as measured on the y axis at right. In the graphs below are shown the time course and approximate extent of the increase in levels of B cells and T cells (gray shading) and the time course of treatments (blue shading). Arrows on the timelines indicate the timing of the intrathecal infusion of AAV-miR-SOD1, the intravenous (IV) administration of methylprednisolone, and the patient's terminal respiratory arrest.

cubic millimeter, and the protein level was 99 mg per deciliter (Table S1). At 12 months after treatment (nearly 18 months after the onset of ALS symptoms), he could propel himself in a wheelchair using the right leg, and vital capacity was reduced to 21% of the predicted value. At 14 months, he regained the ability to extend and flex the fingers of the left hand, a function that had been absent for the previous 20 weeks. Subsequently, he received continuous noninvasive ventilation and died of respiratory arrest 15.6 months after the initiation of treatment and 20.5 months after the onset of ALS symptoms.

The results of autopsy performed 4 hours after death showed a loss of motor neurons in the cervical, thoracic, and left lumbosacral spinal cord but relative sparing of motor neurons in the right lumbosacral spinal cord as gauged visually; this finding corresponded to the preservation of some strength in his right leg (Fig. 1C). The cortical ribbon in the primary motor–sensory cortex was moderately gliotic. Pyramidal neurons had pyknotic nuclei and hypereosinophilic cytoplasm, findings that were consistent with acute hypoxic–ischemic injury. Other regions showed neuronal loss, attributed to the terminal hypoxic–ischemic injury. Immunohistochemical staining for glial fibrillary acidic protein showed gliosis throughout the anterior aspect of the spinal cord, the motor cortex, and the underlying cortical–subcortical junction, with accentuation of this finding in the pyramidal layers of the cortex. Immunostaining for phosphorylated TDP-43 did not reveal cytoplasmic neuronal inclusions in spinal cord or motor cortex, which was consistent with familial ALS caused by *SOD1* mutations. Neurons were depleted in bilateral dorsal-root ganglia, with corresponding pallor of the dorsal columns and a T-lymphocytic infiltrate of some of the proximal nerve roots. Skeletal muscle showed denervation in the left, but not the right, gastrocnemius muscle. Liver findings were compatible with a preceding centrilobular hepatic injury.

On Western immunoblotting, the ratio of SOD1 to actin in a sample of lumbosacral spinal cord obtained from Patient 1 was 0.25, which was more than 90% lower than the level either in five samples obtained from other patients who had ALS with the *SOD1*-A5V mutation (mean [±SD] ratio, 7.57±7.19) or in five control samples (3.79±1.59). Results for the cervical cord were similar to those in five samples obtained from other patients with the *SOD1* mutation; there was a corresponding reduction in SOD1 enzymatic activity in the cervical and lumbosacral spinal cord[12,13] (Fig. 3A and 3B and Table S2). The AAV-miR-SOD1 viral genome was detected in the cervical and lumbosacral spinal cord parenchyma (Fig. S4). As compared with a baseline SOD1 level of 120 ng per milliliter in the CSF, the level was 102 ng per milliliter at 8 weeks and 120 ng per milliliter at 41 weeks (Table S1).

PATIENT 2

Patient 2 was a 56-year-old man with a family history of ALS who had a homozygous missense mutation in *SOD1* that replaced aspartate with alanine at position 91 (D91A–D91A). In the autumn of 2013, he first noted distal weakness in both legs; in November 2017, the examination showed bilateral wrist-extension weakness (MRC grade, 3) and reduced hip-flexion strength (MRC grade, 2). He had Babinski signs and excessively reactive finger jerks to percussion. Sensory function was normal. As a result of the meningoradiculitis that developed after treatment in Patient 1, we aimed to suppress B-cell activity and T-cell function with rituximab (at a dose of 375 mg per square meter of body-surface area), which was initiated in late August 2018 in weekly intravenous infusions for 3 weeks and with intravenous methylprednisolone (at a dose of 125 mg before each dose of rituximab and 1 g on the day of AAV-miR-SOD1 infusion). Beginning at the initiation of study treatment, the patient began receiving daily oral sirolimus (6 mg). The day after treatment, oral prednisone (0.5 mg per kilogram of body weight) was initiated; sirolimus and prednisone were continued for 6 months.

On September 17, 2018, he received an intrathecal infusion of 4.2×10^{14} vector genomes of AAV-miR-SOD1. During the year before therapy, his functional status had been stable, with ALSFRS-R scores averaging close to 28; during testing that was conducted 60 weeks after treatment, the score was 24, signifying worse overall function. For a year before treatment, his slow vital capacity had ranged from 42 to 58% of the predicted value; at 65 weeks after therapy, the value was 62% (Fig. 1A). On the day after treatment and at weeks 12 and 17, he received intravenous immune globulin (at a dose of 0.4 mg per

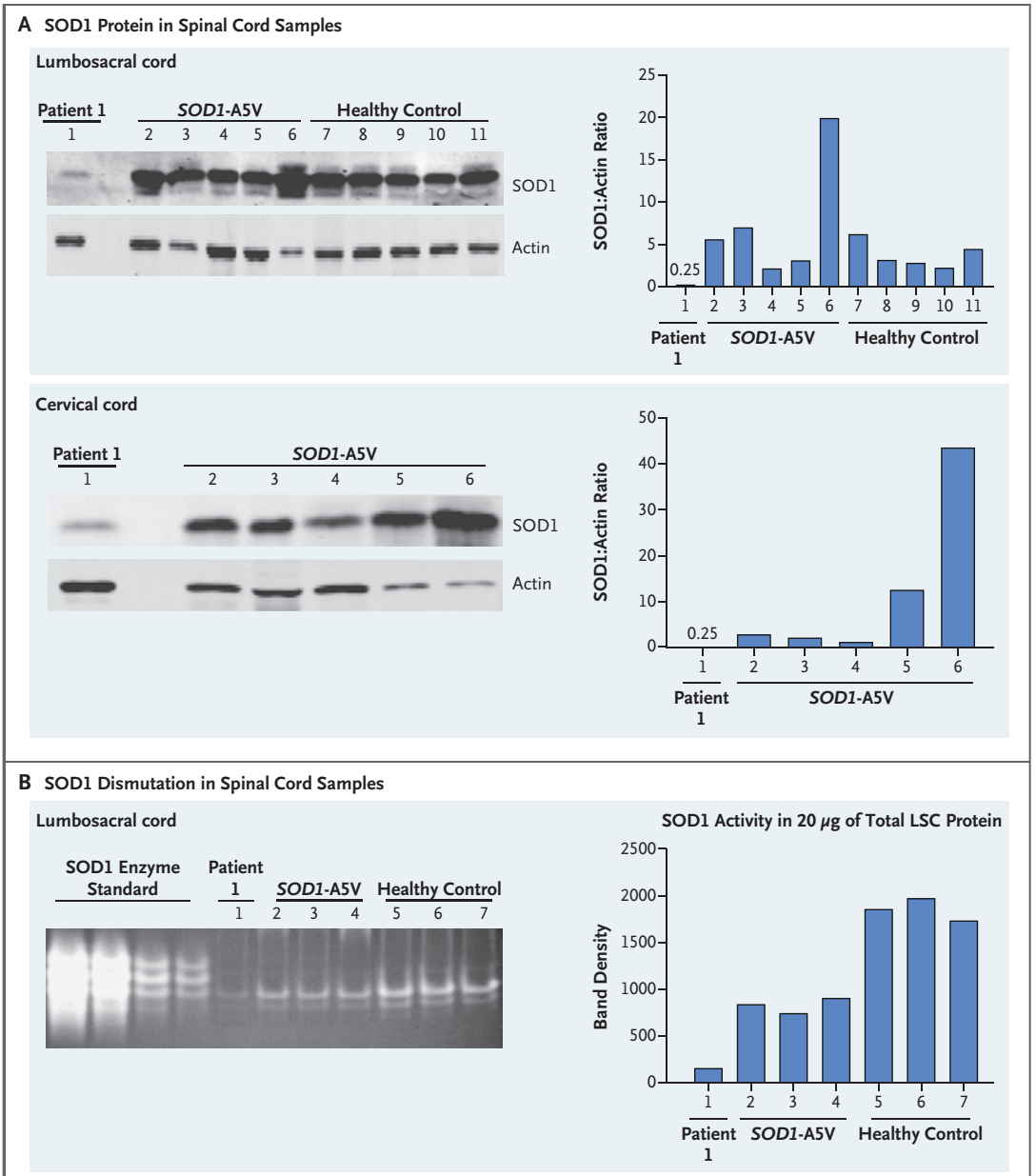

Figure 3. Western Immunoblot Analysis of SOD1 Protein in Spinal Cord.

Panel A shows the results of Western immunoblot analysis of SOD1 and actin proteins (the latter as a control) in samples obtained from Patient 1. The upper portion of the panel shows the results of the analysis of lumbosacral cord (LSC) obtained from Patient 1, from five patients with ALS who had a *SOD1* missense mutation replacing alanine with valine at position 5 (*SOD1*-A5V), and from five controls. The lower portion of the panel shows the results of analysis of cervical cord obtained from Patient 1 and from five patients with *SOD1*-A5V. The histograms at right present the ratios of band densities of SOD1 to actin for each Western blot. Panel B shows the SOD1 dismutation activity in lumbosacral cord obtained from Patient 1, from three patients with *SOD1*-A5V, and from three controls. Gel activity is shown on the left, and gel quantification is shown in the graph on the right. Standard methods were used for Western immunoblotting[12] and for assays of SOD1 enzyme activity.[13]

kilogram) in response to a decrease in the serum IgG to a level of less than 700 mg per deciliter, which had been induced by rituximab. In contrast to the clinical course of Patient 1, the immunosuppressive regimen in Patient 2 blunted the generation of neutralizing antibodies, antiviral antibodies, and T-cell response to the viral capsid (Fig. S2B and S2C). Patient 2 did not have elevated hepatic aminotransferase levels, sensory dysfunction, or CSF pleocytosis. As of May 18, 2020, his disease course was stable, with a functional measure of 24 at 90 weeks after treatment.

DISCUSSION

We designed this study to evaluate the safety of the AAV-mediated silencing of mutated genes in two selected patients with ALS, with descriptive and some objective measures of clinical function and biologic effects of SOD1 levels on tissue and CSF. In Patient 1, a single intrathecal dose of AAV-miR–SOD1 resulted in levels of SOD1 in the spinal cord that were lower than levels in samples obtained from other patients with *SOD1* mutations and from controls. However, the viral vector therapy had no effect on SOD1 levels in the CSF. In this patient, we cannot determine whether the strength in his right leg remained stable or improved slightly after treatment, since that limb had normal strength at the beginning of the study and had been relatively strong throughout his course of treatment. There was similar ambiguity in the interpretation of the corresponding preservation of motor neurons on the right side of the lumbosacral spinal cord (as compared with a lack of preservation on the left side) on autopsy.

At 14 months after the initiation of treatment, he recovered minimal finger extension in the left hand according to visual observation by examiners, but other clinical features and measures of vital capacity declined, as is typical in patients with ALS. We cannot conclude that suppression of SOD1 played a role in his clinical course, since such improvements in function may have reflected recovery from meningoradiculitis. The experience with this patient indicates that intrathecal infusion of this viral vector can trigger an adverse inflammatory response (Table S2), as has been reported in some studies after the intravenous administration of AAV9 in animals.[14] In Patient 2, we attenuated this effect with immunosuppression,[15] although viral vector therapy provided him with no clinical benefit. His functional status and vital capacity were relatively stable during a 60-week period, a course that could be typical of the slow disease progression in patients with his *SOD1* genotype, so no clinical conclusions can be made about the treatment effects.[16]

These results suggest that the intrathecal infusion of AAV-delivered microRNAs can be accomplished but may require the use of immunosuppression. Although attenuation of SOD1 protein levels in CSF has been seen with antisense oligonucleotides,[17] we did not find this result at 2 weeks after the initiation of treatment in our study. A theoretical advantage of viral vector–mediated gene suppression is the potential for the sustained effect of a single dose of therapy, which is balanced by the possibility that viral vectors may mediate long-lasting adverse effects. Additional studies are required to determine the results of this method in a larger number of patients who have ALS with *SOD1* mutations.

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

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