

# Safety of intrathecal autologous adipose-derived mesenchymal stromal cells in patients with ALS



Nathan P. Staff, MD,  
PhD  
Nicolas N. Madigan,  
MBBCh, PhD  
Jonathan Morris, MD  
Mark Jentoft, MD  
Eric J. Sorenson, MD  
Greg Butler, BSc  
Dennis Gastineau, MD  
Allan Dietz, PhD\*  
Anthony J. Windebank,  
MD\*

Correspondence to  
Dr. Staff:  
staff.nathan@mayo.edu

## ABSTRACT

**Objective:** To determine the safety of intrathecal autologous adipose-derived mesenchymal stromal cell treatment for amyotrophic lateral sclerosis (ALS).

**Methods:** Participants with ALS were enrolled and treated in this phase I dose-escalation safety trial, ranging from  $1 \times 10^7$  (single dose) to  $1 \times 10^8$  cells (2 monthly doses). After intrathecal treatments, participants underwent standardized follow-up, which included clinical examinations, revised ALS Functional Rating Scale (ALSFRS-R) questionnaire, blood and CSF sampling, and MRI of the neuroaxis.

**Results:** Twenty-seven patients with ALS were enrolled and treated in this study. The safety profile was positive, with the most common side effects reported being temporary low back and radicular leg pain at the highest dose level. These clinical findings were associated with elevated CSF protein and nucleated cells with MRI of thickened lumbosacral nerve roots. Autopsies from 4 treated patients did not show evidence of tumor formation. Longitudinal ALSFRS-R questionnaires confirmed continued progression of disease in all treated patients.

**Conclusions:** Intrathecal treatment of autologous adipose-derived mesenchymal stromal cells appears safe at the tested doses in ALS. These results warrant further exploration of efficacy in phase II trials.

**Classification of evidence:** This phase I study provides Class IV evidence that in patient with ALS, intrathecal autologous adipose-derived mesenchymal stromal cell therapy is safe.  
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## GLOSSARY

**ALS** = amyotrophic lateral sclerosis; **ALSFRS-R** = revised ALS Functional Rating Scale; **MSC** = mesenchymal stromal cell.

Amyotrophic lateral sclerosis (ALS) is a devastating progressive paralytic disorder due to the death of motor neurons in the brain and spinal cord. The average survival after the onset of symptoms is 3 years, and there are no adequate therapies.<sup>1</sup> The pathomechanisms of ALS are complex and insufficiently understood.<sup>2</sup>

There has been increased interest in the potential of cell-based therapies to treat ALS. One cell type, the mesenchymal stromal cell (MSC), has been suggested as a possible therapy. MSCs secrete multiple neurotrophic growth factors and cytokines and have the ability to modulate the immune system, all of which may influence the progression of ALS.<sup>3</sup>

We have now completed a phase I dose-escalation safety clinical trial that used intrathecal delivery of adipose-derived autologous MSCs to treat advanced ALS.

**METHODS** Criteria for enrollment in the study included the diagnosis of definite ALS based on El Escorial criteria, a vital capacity of  $>65\%$  predicted for age and biometrics, no major medical comorbidities, and symptoms of weakness lasting  $>1$  year but  $<2$  years. Patients were sequentially enrolled into 5 groups of escalating MSC dose: group 1 ( $1 \times 10^7$ , one injection), group 2 ( $5 \times 10^7$ , one injection), group 3 ( $5 \times 10^7$ , 2 monthly injections), group 4 ( $1 \times 10^8$ , one injection), and group 5 ( $1 \times 10^8$ , 2 monthly injections).

Enrolled participants underwent an open adipose biopsy of abdominal fat  $\geq 8$  weeks before MSC therapy. After isolation from the adipose biopsy, MSCs were expanded and cryopreserved in the Mayo Clinic Human Cellular Therapy Laboratory (e-Methods at Neurology.org). MSCs were suspended in lactated Ringer solution and infused intrathecally after a standard lumbar puncture. Four weekly follow-up visits took place after treatment and included review of symptom diary, vital signs, revised ALS Functional Rating

\*These authors contributed equally to this work.

From the Departments of Neurology (N.P.S., N.N.M., E.J.S., A.J.W.), Radiology (J.M.), and Laboratory Medicine and Pathology (M.J., G.B., D.G., A.D.), Mayo Clinic, Rochester, MN.

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Supplemental data  
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**Table** Demographics and study data

Participant	Dose group	Age, y	Sex	Site of onset	ALSFRS-R at enrollment	Adverse events	MRI lumbosacral spine changes	Peak CSF protein, mg/dL	Peak CSF nucleated cells, cells/ $\mu$ L	Trough CSF glucose, mg/dL	Lumbosacral pain	Subjective benefit	Follow-up, wk
1-1	$1 \times 10^7$	62	F	Spinal	30	No	Not done	64	4	61	No	No	4 <sup>a,b</sup>
1-2	$1 \times 10^7$	63	F	Spinal	29	No	No	25	1	51	No	No	105 <sup>a</sup>
1-3	$1 \times 10^7$	45	M	Spinal	41	No	No	49	2	55	No	Yes	108
1-4	$1 \times 10^7$	50	M	Spinal	31	No	Yes	87	4	51	No	Yes	47 <sup>a</sup>
1-5	$1 \times 10^7$	50	M	Spinal	38	No	No	69	2	52	No	Yes	41 <sup>a,c</sup>
2-1	$5 \times 10^7$	69	M	Spinal	39	No	Yes	574	10	46	No	No	105
2-2	$5 \times 10^7$	47	M	Spinal	30	No	Yes	93	2	46	No	No	48 <sup>a,c</sup>
2-3	$5 \times 10^7$	56	F	Spinal	42	No	No	49	7	42	No	Yes	108 <sup>a,c</sup>
2-4	$5 \times 10^7$	42	M	Spinal	27	HA	Yes	88	13	44	No	Yes	35 <sup>c</sup>
2-5	$5 \times 10^7$	58	M	Spinal	43	No	No	44	2	61	No	Yes	41 <sup>a</sup>
3-1	$5 \times 10^7 \times 2$	58	F	Bulbar	38	No	Yes	182	7	36	No	Yes	87 <sup>a</sup>
3-2	$5 \times 10^7 \times 2$	52	M	Spinal	41	No	Yes	62	18	52	No	Yes	84 <sup>a</sup>
3-3	$5 \times 10^7 \times 2^d$	55	M	Spinal	40	No	Yes	173	2	54	No	No	54 <sup>a,b</sup>
3-4	$5 \times 10^7 \times 2$	55	M	Spinal	47	No	No	80	2	54	No	No	79
3-5	$5 \times 10^7 \times 2$	61	F	Bulbar	41	No	Yes	36	5	51	No	Yes	86
3-6	$5 \times 10^7 \times 2^e$	73	M	Spinal	42	No	Yes	609	3	60	No	No	39 <sup>a,b</sup>
3-7	$5 \times 10^7 \times 2$	40	F	Spinal	30	No	Yes	71	5	40	No	Yes	41 <sup>c</sup>
4-1	$1 \times 10^8$	39	M	Spinal	39	No	Yes	141	7	37	No	No	51
4-2	$1 \times 10^8$	63	F	Spinal	32	LBP/radic	Yes	251	20	27	Yes	Yes	40 <sup>c</sup>
4-3	$1 \times 10^{8f}$	56	F	Spinal	30	No	No	64	2	58	No	No	30
4-4	$1 \times 10^8$	56	F	Bulbar	38	LBP/radic	Yes	79	9	44	Yes	No	31 <sup>a,b</sup>
4-5	$1 \times 10^8$	68	F	Bulbar	39	LBP/radic	Yes	153	11	53	Yes	Yes	26
5-1	$1 \times 10^8 \times 2$	59	F	Spinal	45	LBP/radic	Yes	171	17	41	Yes	Yes	8
5-2	$1 \times 10^8 \times 2$	54	M	Bulbar	31	Fever, LBP/radic	Yes	197	65	31	Yes	Yes	19
5-3	$1 \times 10^8 \times 2$	36	M	Spinal	39	LBP/radic	Yes	879	29	46	Yes	Yes	15
5-4	$1 \times 10^8 \times 2$	62	M	Bulbar	42	LBP/radic	Yes	338	33	31	Yes	Yes	16
5-5	$1 \times 10^8 \times 2$	60	F	Bulbar	41	LBP/radic	Yes	1,250	40	32	Yes	Yes	16

Abbreviations: ALSFRS-R = ALS Functional Rating Scale; HA = headache; LBP/radic = low back and radicular leg pain.

<sup>a</sup>Death.

<sup>b</sup>Autopsy.

<sup>c</sup>Lost to follow-up.

<sup>d</sup>Protocol deviation: mesenchymal stromal cell (MSC) first dose  $4 \times 10^7$ , second dose  $3 \times 10^7$ .

<sup>e</sup>Protocol deviation: MSC first dose  $3 \times 10^7$ .

<sup>f</sup>Protocol deviation: MSC dose  $6.3 \times 10^7$ .

Scale (ALSFRS-R) questionnaire, neurologic examination, physical examination, blood sample (weeks 1 and 3), CSF samples (weeks 1 and 4), and gadolinium-enhanced MRI of the brain and spinal cord (week 3). In participants receiving the 2 monthly treatments, the weekly follow-up visits were repeated for a total of 8 weeks. Subsequently, participants were followed up every 3 months until 2 years, death, or loss to follow-up.

**RESULTS** After providing Institutional Review Board–approved informed consent, 27 patients with clinically definite ALS by El Escorial criteria were enrolled and treated in this dose-escalation safety clinical trial (table). The median age was 57 years

(range 36–75 years), and the ratio of male to female patients was 15:12. The median ALSFRS-R at enrollment was 39 (range 27–47). Twenty of 27 patients had spinal-onset ALS, and 7 of 27 had bulbar onset.

Adverse events that were considered to be possibly, probably, or definitely related to intrathecal MSCs were observed in a dose-dependent fashion. Participant 2-4 had 24 hours of headache after treatment (not position dependent). Eight of the 10 participants receiving  $1 \times 10^8$  MSC dose developed low back and leg pain that began within a week of

treatment, peaked in the first 3 weeks, and then resolved or became very mild (1 of 10 on visual analog scale). The pain was exacerbated by the straight leg raise test and exhibited variable intensity (2–8 of 10 on visual analog scale) and duration (range 42–77 days) among the participants. Analgesics were used during pain in 5 of 8 patients, including nonsteroidal anti-inflammatory drugs (5), gabapentin (3), and opioids (2). In participant 5-2, the pain led to postponement of the second treatment by 2 months until pain had resolved. The pain recurred on the second injection, lasting 2 months.

Although the study was not designed for efficacy, ALSFRS-R questionnaires were performed throughout the study and showed progression in all patients that did not appear, and was not reported by any patients, to be more rapid than before therapy (figure 1). Notably, 17 of 29 participants reported specific mild temporary subjective clinical improvements (typically lasting <2 months), which were reported (with overlap) as improved bulbar function (n = 8), increased limb strength (n = 5), decreased fasciculations (n = 4), decreased stiffness (n = 4), and improved energy (n = 4). Because this trial was not blinded or placebo controlled, these effects cannot be interpreted as a positive treatment response to MSC treatment.

Lumbar punctures were performed at weeks 1 and 4 after intrathecal MSC treatment. Dose-dependent alterations of CSF parameters were observed. In aggregate within the different doses, CSF protein increased from baseline (mean = 56.4 mg/dL, SEM = 4.0 mg/dL) to week 1 (mean = 93.4 mg/dL, SEM = 18.7 mg/dL) and week 4 (mean = 189.2 mg/dL, SEM = 111.0 mg/dL) after intrathecal MSCs administration. There was also an increase in CSF nucleated cells (monocytes) from baseline (1.2 cells/ $\mu$ L, SEM

0.3 cell/ $\mu$ L) to week 1 (mean = 9.2 cells/ $\mu$ L, SEM = 2.5 cells/ $\mu$ L) that began to decrease by week 4 (mean = 4.7 cells/ $\mu$ L, SEM = 1.5 cells/ $\mu$ L). Glucose decreased slightly between baseline and weeks 1 and 4. No abnormal CSF oligoclonal bands or neoplastic cytology was observed in any sample. Grouped data by dose level are reported in figure 2, and individual data are included in the table.

MRI of the brain and spinal cord was completed, when tolerated, at week 3 after intrathecal MSCs. Abnormalities were observed in the lumbosacral nerve roots in 1 of 5 patients receiving  $1 \times 10^7$ , 9 of 12 receiving  $5 \times 10^7$ , and 9 of 10 in the  $1 \times 10^8$  dose group. In these cases, the primary finding was lumbosacral nerve root thickening with clumping and gadolinium enhancement, reminiscent of arachnoiditis, which increased in severity with increasing dose (figure 2). In participant 2-1, the first participant whom we observed to have clear lumbosacral nerve root abnormalities, repeat MRI was performed at week 22 after treatment and was unchanged. CSF in participant 2-1 was also repeated at week 22 and normalized.

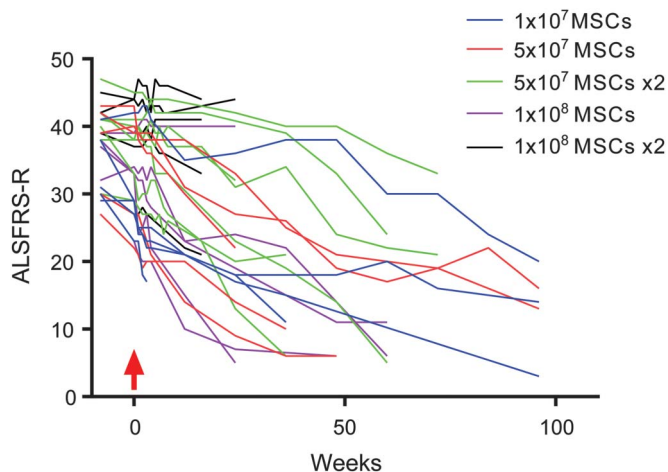
Four patients who died in this study underwent autopsy at a range of 31 days to 54 weeks after MSC treatment (3 of whom had abnormalities on MRI lumbosacral spine). Gross pathology did not reveal any signs of tumor formation or frank arachnoiditis in any of these cases.

**DISCUSSION** Overall, intrathecal administration of MSCs in patients with ALS was safe. We observed dose-dependent changes in MRI of lumbosacral spine, CSF parameters, and temporary lumbosacral-radicular pain, all of which were tolerable. We postulate that this temporary pain relates to nerve root irritation and inflammation from MSC administration. Notably, in the 4 patients who underwent autopsies, there were no signs of tumor formation, which has been a concern with cell-based therapies. Studies are ongoing to investigate autopsy material for microscopic signs of MSC engraftment or neuroinflammation.

This phase I clinical trial of intrathecal adipose-derived MSC therapy in patients with definite ALS by El Escorial criteria corroborates other studies that have demonstrated safety of intrathecal bone marrow-derived MSCs in ALS.<sup>4-7</sup> Although still debated, MSCs from adipose and bone marrow have similar properties, and adipose harvesting is a simpler, well-tolerated procedure. These safety data support the exploration of adipose-derived MSCs in other neurologic conditions.

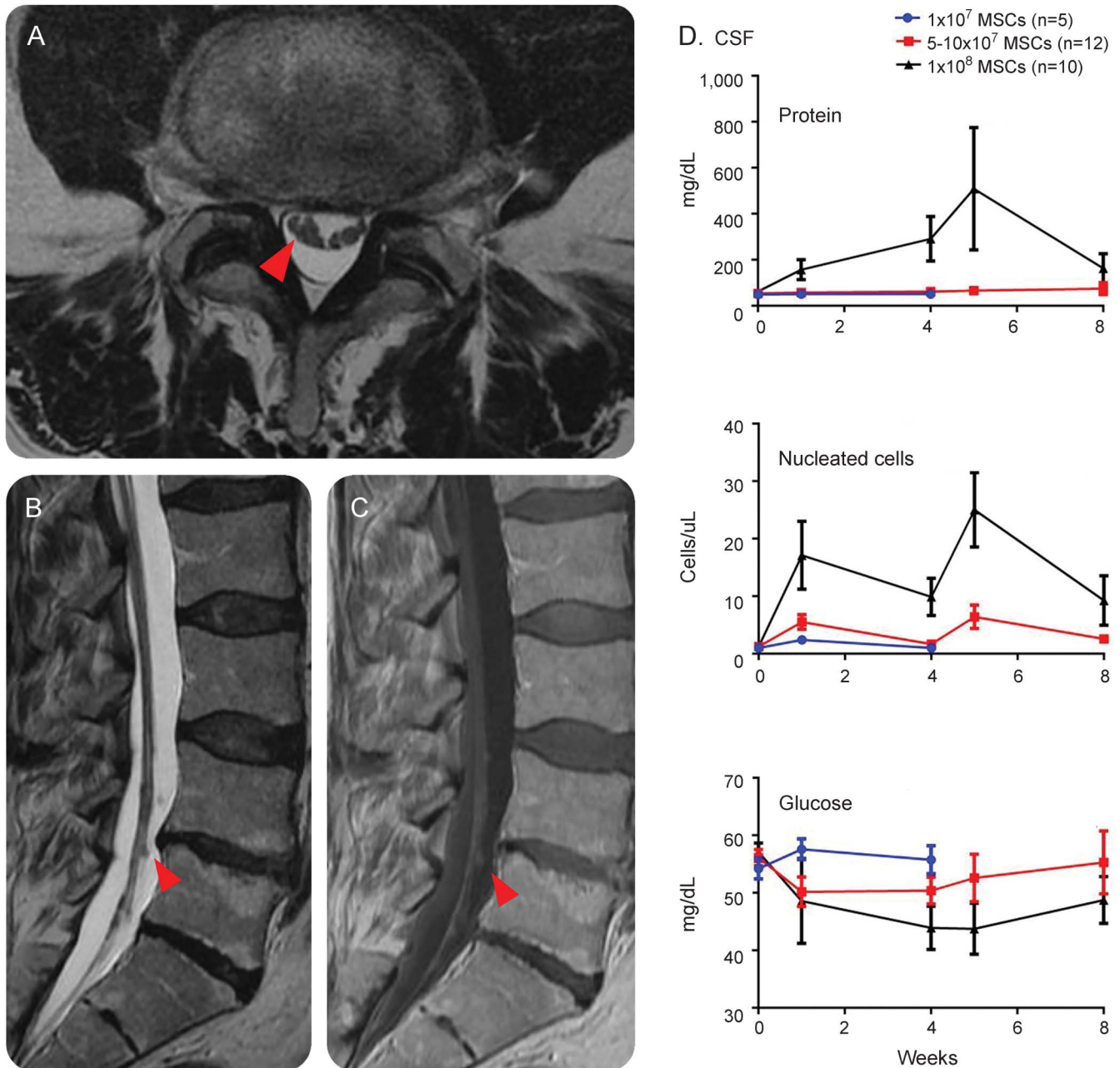
Our data do not directly address the efficacy of intrathecal MSC therapy in ALS; however, we feel that the excellent safety profile justifies further investigation of this product. Notably, while we report

**Figure 1** ALS Functional Rating scale-revised (ALSFRS-R)



ALSFRS-R demonstrated progression during the duration of follow-up. Mesenchymal stromal cell (MSC) treatment occurred at week 0 (red arrow).

**Figure 2** MRI and CSF findings in patients receiving intrathecal mesenchymal stromal cells (MSCs)



Nodularity of lumbosacral nerve roots (red arrows) was observed in patients 3 weeks after treatment, as shown on MRIs from patient 2-1 (A, axial T2-weighted sequence; B, sagittal T2-weighted sequence; and C, sagittal gadolinium-enhanced T1-weighted sequence). (D) Elevations in CSF protein and nucleated cells were seen in a dose-dependent fashion.

anecdotal mild temporary subjective clinical improvements in our treatment regimen, we did not see miraculous improvements in or cessation of progression in any patients. These data may temper the sometimes irrationally exuberant enthusiasm for this therapy modality. Future studies are required to determine the efficacy and whether earlier intervention or additional MSC treatments are required to produce meaningful results. In this small phase I study,  $1 \times 10^8$  MSCs were tolerated, yet this dose also caused more painful adverse effects. Our planned

multidose phase II study initiates treatment at  $1 \times 10^8$  MSCs but incorporates an algorithm that allows lower doses when there is protracted pain.

#### AUTHOR CONTRIBUTIONS

Nathan P. Staff, MD, PhD: study concept and design, obtaining funding, acquisition of data, study supervision and coordination, analysis and interpretation, drafting and revising the manuscript for content. Nicolas N. Madigan, MBBCh, PhD: acquisition of data, analysis and interpretation, revising the manuscript for content. Jonathan Morris, MD: analysis and interpretation, revising the manuscript for content. Mark Jentoft, MD, and Eric J. Sorenson, MD: acquisition of data, analysis and interpretation, revising the manuscript for content. Greg Butler, BSc: contribution of vital

tools, acquisition of data, revising the manuscript for content. Dennis Gastineau, MD: study supervision and coordination, analysis and interpretation, revising the manuscript for content. Allan Dietz, PhD, and Anthony J. Windebank, MD: study concept and design, obtaining funding, acquisition of data, study supervision and coordination, analysis and interpretation, revising the manuscript for content.

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## DISCLOSURE

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been disclosed to and are managed by the Mayo Clinic Conflict of Interest Board and are included here as directed by them. A. Windebank is a site principal investigator for the ALS clinical trial from BrainStorm Cell Therapeutics. Go to [Neurology.org](http://Neurology.org) for full disclosures.

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