

# Transplantation of spinal cord–derived neural stem cells for ALS

## Analysis of phase 1 and 2 trials



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### ABSTRACT

**Objective:** To test the safety of spinal cord transplantation of human stem cells in patients with amyotrophic lateral sclerosis (ALS) with escalating doses and expansion of the trial to multiple clinical centers.

**Methods:** This open-label trial included 15 participants at 3 academic centers divided into 5 treatment groups receiving increasing doses of stem cells by increasing numbers of cells/injection and increasing numbers of injections. All participants received bilateral injections into the cervical spinal cord (C3-C5). The final group received injections into both the lumbar (L2-L4) and cervical cord through 2 separate surgical procedures. Participants were assessed for adverse events and progression of disease, as measured by the ALS Functional Rating Scale–Revised, forced vital capacity, and quantitative measures of strength. Statistical analysis focused on the slopes of decline of these phase 2 trial participants alone or in combination with the phase 1 participants (previously reported), comparing these groups to 3 separate historical control groups.

**Results:** Adverse events were mostly related to transient pain associated with surgery and to side effects of immunosuppressant medications. There was one incident of acute postoperative deterioration in neurologic function and another incident of a central pain syndrome. We could not discern differences in surgical outcomes between surgeons. Comparisons of the slopes of decline with the 3 separate historical control groups showed no differences in mean rates of progression.

**Conclusions:** Intraspinal transplantation of human spinal cord–derived neural stem cells can be safely accomplished at high doses, including successive lumbar and cervical procedures. The procedure can be expanded safely to multiple surgical centers.

**Classification of evidence:** This study provides Class IV evidence that for patients with ALS, spinal cord transplantation of human stem cells can be safely accomplished and does not accelerate the progression of the disease. This study lacks the precision to exclude important benefit or safety issues. *Neurology*® 2016;87:392-400

### GLOSSARY

**AE** = adverse event; **ALS** = amyotrophic lateral sclerosis; **ALSFRS-R** = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; **DSMB** = Data Safety and Monitoring Board; **FDA** = US Food and Drug Administration; **FVC** = forced vital capacity; **HSSC** = human spinal cord–derived neural stem cell; **POD** = postoperative day.

There are few disease-modifying therapeutic options for people with amyotrophic lateral sclerosis (ALS). Supportive preclinical data in ALS rodents show that transplantation of human spinal cord–derived neural stem cells (HSSCs) into the ventral horn of the spinal cord delays the onset of ALS and improves animal survival.<sup>1–4</sup> We embarked on a program in humans to test whether injection of HSSCs directly into the lumbar and cervical segments of the spinal cord is safe, with the ultimate goal of testing in future studies whether this approach can slow or stop disease progression. The results of our phase 1 trial have been reported,<sup>5,6</sup> and we now have completed a phase 2, dose-escalation study in 15 additional participants. The focus of this trial was 3-fold:

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(1) test the safety and tolerability of delivering increasing numbers of cells by both increasing the concentration cells in each injection and increasing the number of injections into the spinal cord; (2) extend the program from a single-center, single-surgeon study, to a 3-center, 3-surgeon study; and (3) evaluate which outcome measures optimally measure stem cell efficacy. The expansion of the trial to 2 additional centers anticipates the need to test whether this highly invasive surgical intervention can be safely performed outside our one center. This will be necessary for a sizable therapeutic efficacy trial as well as to provide this treatment to a large number of patients should it prove successful in ameliorating the course of disease; it will also be used to provide preliminary estimates for planning future efficacy trials.

**METHODS** **Standard protocol approvals, registrations, and patient consents.** The study protocol was approved by the US Food and Drug Administration (FDA), and institutional review boards from each center approved the study protocols. All patients signed informed consent documents stating clearly the risks of participating in the study, that the trial was designed to test the safety of the treatment, and that there was no expectation of clinical benefit. The trial was registered in ClinicalTrials.gov as NCT 01730716.

The phase 1 patients were all treated at Emory University, and the initial results of that trial are published.<sup>5,6</sup> The 3 participating centers for phase 2 were Emory University, Atlanta (7 participants), University of Michigan, Ann Arbor (6 participants), and the Massachusetts General Hospital, Boston (2 participants). The detailed inclusion criteria are presented in table e-1 on the *Neurology*<sup>®</sup> Web site at Neurology.org. Briefly, participants with ALS had to be within 24 months of symptom onset at the time of the screening visit and had to undergo surgery not more than 36 months after symptom onset. All participants were ambulatory with some extremity weakness but not less than antigravity strength, with a seated forced vital capacity (FVC) of  $\geq 60\%$  of normal predicted values. Weakness of neck extensor muscles was exclusionary to avoid the possibility of neck instability after multilevel laminectomies.

Participants were recruited into 5 successive groups of increasing doses, with 3 participants in each group. The demographic characteristics and baseline clinical measures for the 15 phase-2 participants plus the 9 phase-1 participants are presented in table 1. The numbers of injections ranged from 10 to 40, and the numbers of cells injected ranged from 2 million to 16 million. The specifics for the doses of each group are presented in table 2.

As an early-phase trial, the primary research questions were focused on the safety of transplanting increasing doses of HSSCs into the spinal cords of people with ALS, and the ability to expand this procedure to multiple institutions. There was no contemporaneous control group and the study was not powered to show therapeutic efficacy, and thus meets Class IV evidence.

**Surgical procedure.** The surgical transplantation procedure, including the design of the injection platform, was developed by one of the participating surgeons (N.M.B.).<sup>7</sup> Two surgeons

(P.P. and L.B.) were trained by N.M.B., first by performing a series of transplantation procedures on minipigs,<sup>8</sup> assuring that the pigs recovered to their preoperative baseline, and then by observation of N.M.B. performing the procedure on a person with ALS. N.M.B. also traveled to each site to serve as a reference during the first surgery done by each of the other surgeons.

The details of the surgical procedure are published<sup>9,10</sup> (including video of the procedure). Each injection contained the prescribed dose of the cell suspension in a 10- $\mu$ L volume. Group A received 5 injections on each side of the cervical cord, and groups B through D received 10 injections on each side. Group E underwent 2 procedures about 1 month apart, first with 10 injections on each side of the lumbar cord and then 10 on each side of the cervical cord. All injections were spaced 4 mm apart.

As reported for the phase 1 study,<sup>5,6</sup> participants received an immunosuppression regimen of basiliximab (1 dose during surgery, second dose on postoperative day [POD] 3 or 4), prednisone for 1 month following surgery, and tacrolimus and mycophenolate mofetil continued as long as tolerated.

**Assessment of safety.** Participants are followed postoperatively at 2 and 4 weeks, and then at months 3, 6, 9, 12, 15, 18, 21, and 24 and then at every 6 months thereafter until death. Participants are evaluated for adverse events (AEs), clinical laboratory tests, and physical examination. This report includes data on all patients up to 9 months. Close attention was given to changes in neurologic function, including new, or acceleration of, weakness and respiratory compromise as documented by the neurologic examination and serial measures of the ALS Functional Rating Scale–Revised (ALSFRRS-R),<sup>11</sup> FVC, and grip strength. MRI focusing on the surgical site was done in the immediate postoperative period and at months 1, 6, and 12 after surgery. Safety endpoints also included complications associated with immunosuppressive therapy. All AEs were reported to the Data Safety and Monitoring Board (DSMB) who adjudicated all AEs in compliance with FDA “Guidance for Clinical Trial Sponsors” (OMB control number 0910-0581). Trial continuation and progression to successive cohorts was the decision of the DSMB.

**Identification of control groups.** These were open-label trials and consequently we were unable to compare outcomes directly with a randomized comparison group. Thus, we decided to further address the question of safety by generating separate comparison groups from the dataset available from the placebo arm of the completed trial of ceftriaxone in ALS<sup>12</sup> (provided by the NEALS consortium) and from the ProACT dataset,<sup>13</sup> which contains the data from control arms of multiple completed ALS trials. In addition, we searched the Emory ALS Center database for patients matching the clinical characteristics of each of the participants in the phase 1 and phase 2 trials, controlling as closely as possible for age at onset of symptoms, sex, and time from onset to “intervention,” which for trial participants was the date of surgery and for database patients the date of first clinical measurements. We excluded the first 6 participants from the phase 1 trial because they were particularly advanced in their disease (3 were maintained on mechanical ventilators), making them obvious outliers.<sup>5</sup> The analysis included the remaining 9 participants from phase 1 and all 15 participants in phase 2. Analyses included comparisons to controls regarding slopes of decline over the first 9 months for ALSFRRS-R, FVC % predicted, and grip strength (available only for the ceftriaxone dataset) with either all 24 participants (phases 1 and 2), or with only the 15 phase 2 participants.

**Statistical analysis.** We performed longitudinal analysis of the outcomes by comparing mean slopes of the trial participants to

**Table 1** Patient demographics for phase 1 and phase 2 participants

Phase/ cohort	Patient no.	Sex	Age at onset, y	Site of onset	Age at surgery, y	Disease duration at surgery, mo	ALSFRS-R score at baseline	Seated FVC % predicted at baseline	GST right at baseline, lb	GST left at baseline, lb
1/B	207	M	57.4	Limb	59	19.5	35	89	61	45
	208	M	35.5	Limb	41.1	68.1	29	74	19	26
	209	M	53.3	Limb	54.6	15.7	31	97	11	25
1/C	210	M	37.3	Limb	48.9	141.4	35	83	50	47
	211	M	37.7	Limb	39.3	19.0	30	96	62	44
	212	M	62.1	Limb	65.1	36.4	38	125	85	75
1/D	213	M	47.2	Limb	50.3	37.6	31	88	55	62
	214	F	52.5	Bulbar	54.3	21.8	29	83	68	25
	215	F	33.5	Limb	35.2	20.4	40	103	81	62
2/A	301	F	57.4	Limb	58.2	10.2	34	86	65	22
	302	M	46.0	Limb	46.6	7.4	39	93	67	85
	303	M	40.7	Limb	42.3	19.3	40	80	120	110
2/B	304	F	39.6	Limb	41.5	23.3	31	85	38	65
	305	M	57.0	Limb	57.5	5.5	44	92	74	103
	306	M	45.3	Limb	46.5	13.9	38	70	10	5
2/C	307	M	65.0	Limb	66.5	18.5	35	110	65	65
	308	F	49.4	Bulbar	51.0	19.5	31	70	6	11
	309	M	27.7	Limb	29.4	20.2	38	93	36	25
2/D	310	M	51.5	Limb	52.4	11.0	31	78	0	0
	311	M	42.1	Limb	43.6	17.2	41	75	46	26
	312	M	47.0	Limb	49.0	23.6	28	102	11	3
2/E <sup>a</sup>	313	M	58.3	Limb	59.2	11.6	39	101	5	58
	314	M	34.0	Limb	35.3	15.3	47	102	52	39
	315	M	60.1	Limb	61.4	15.8	44	102	40	42

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; FVC = forced vital capacity; GST = grip strength. Cohort 1/E comprises the same patients as cohort 1/C.

<sup>a</sup>Data for cohort 2/E are for the first surgery.

each control group using linear mixed models analysis of variance.<sup>14</sup> Briefly, for each outcome, we modeled response as a linear function of time, with terms to allow testing for equal y-intercepts and equal slopes for control and trial participants. For each

patient, time is the days since (first) surgery. Detailed statistical methods are presented in the supplemental material.

In addition to the formal linear mixed models analysis, we conducted an exploratory analysis of the estimated slopes. We

**Table 2** Dosing scheme for surgical transplantation of human spinal cord-derived neural stem cells from phase 1 and 2 studies

Group	Surgical region	No. of injections	Cells/injection	Total dose, in millions
1/B	L2-L4	5 (unilateral)	100,000	0.5
1/C	L2-L4	10 (5 bilateral)	100,000	1
1/D	C3-C5	5 (unilateral)	100,000	0.5
1/E	C3-C5 (C group redosed)	5 (unilateral)	100,000	1.5
A	C3-C5	10 (5 bilateral)	200,000	2
B	C3-C5	20 (10 bilateral)	400,000	4
C	C3-C5	20 (10 bilateral)	600,000	6
D	C3-C5	20 (10 bilateral)	800,000	8
E	L2-L4, then C3-C5	20 (10 bilateral) × 2	800,000	16

obtained 95% confidence limit estimates for the means of the slopes for the various control groups. We then estimated the slope of decline for each outcome for each participant and classified these slopes in relation to the confidence limit estimates as below the lower limit, between the lower and upper limits, or above the upper limit.

**RESULTS** AEs occurring in phase 1 are reported in our 2 previous publications.<sup>5,6</sup> Table 3 summarizes the AEs occurring in phase 2, including the possible relationship to surgery, the surgical device, the stem cells, and immunosuppressive drugs as determined by the site investigator. There were 2 participant deaths in the phase 2 trial before 270 days that were attributed to disease progression. Thirty-day surgical outcomes revealed no wound dehiscence, wound infections, CSF leaks, meningitis, or hematoma formations. Of 165 reported AEs, 14 were thought possibly or probably related to the stem cells, and none were considered definitely related to the stem cells. The majority of AEs were related to surgery, including incisional pain and transient paresthesias, with the second most common events related to the immunosuppressive medications. Two participants stopped tacrolimus and mycophenolate for the problems of headache (1) and diarrhea (1). Two other participants stopped only tacrolimus because of new-onset diabetes mellitus, a known toxicity of tacrolimus. One patient was treated for duodenitis. Two participants experienced pulmonary embolism, which is an uncommon but known risk in patients with ALS as well as in patients undergoing spine surgery.<sup>15,16</sup> The relative numbers of AEs and serious AEs did not differ significantly between sites (table 3), although the relatively few events that did occur makes quantitative comparisons difficult.

Overall, the majority of participants had uneventful surgical courses and were discharged from hospital by POD 4. However, severe complications related to trial participation were experienced by 2 participants, and require special mention here. Participant 312 was in cohort D and received 10 bilateral cervical injections with 400,000 cells/injection. Surgery was deemed “uncomplicated,” but myoclonus in the lower extremities was noted in the immediate postoperative period. On POD 2, he had reduced sensation to pinprick below the neck, severe neck pain, a burning sensation in both arms, and episodes of myoclonus in both legs. Dexamethasone ameliorated the myoclonus and reduced the pain, which was controlled with morphine, although the loss of sensation persisted. On POD 6, he was noted to have significant loss of strength in bilateral lower extremities; cervical MRI scan showed spinal cord edema. He was treated with dexamethasone and on POD 7 underwent a C6 laminectomy and dural graft to relieve external pressure on the cord. Following this

second surgery, there was some reduction of pain, although the sensory and motor abnormalities did not resolve. He was transferred to a rehabilitation facility on POD 22. As compared to his preoperative neurologic function, there was partial loss of sensation below the neck with persistent burning pain in the arms, partial loss of bowel and bladder control, and he was unable to walk independently. He spent 75 days in the rehabilitation hospital, at which time he could support his weight and assist with transfers, and bowel and bladder dysfunction had resolved. He continued to improve, and 5 months after surgery, he could walk about 120 yards with a walker, but he continued to report moderate neck pain treated with analgesics and physical therapy.

It was clear that the neurologic deterioration was due to the acute spinal cord swelling, but investigations into the cause of the swelling were inconclusive. There was no evidence of infection, and immunologic studies did not show markers of rejection of the transplanted cells. Review of the intraoperative videos by the 2 other participating surgeons identified some variations in the surgical procedure but it was not clear that these were responsible for the adverse outcome. There was also speculation that the presence of premorbid spinal stenosis might have rendered the cord more vulnerable to compressive injury during the injection procedure.

Participant 315 was in cohort E and underwent lumbar and then cervical procedures with 10 bilateral injections of 400,000 cells/injection. Of note is that this participant had a history of “transverse myelitis” diagnosed 25 years previously with full neurologic recovery, and also a diagnosis of ulcerative colitis treated with immunosuppressant agents. The surgical procedures were accomplished without apparent complications. Following the lumbar surgery, there was mild neuropathic pain in the left thigh that resolved without specific treatment by POD 12. Cervical surgery occurred 6 weeks after the lumbar surgery. The neuropathic thigh pain returned approximately 2 months after the cervical surgery. Five months after cervical surgery, there was onset of neuropathic pain in both forearms. The pain was assessed as “severe” and did not respond to physical therapy or oral analgesics. Examination showed allodynia in the left thigh and the patient reported “shooting pains” that began in the thumb and traveled up the arm, occurring “hundreds of times a day.” There were no motor deficits other than those associated with his ALS. Femoral and radial nerve blocks did not relieve the pain and it was concluded that the pain was spinal in origin. The participant continues to be followed in a pain clinic and requires narcotics to control his pain.

Imaging of the surgical regions and CSF studies did not show evidence of swelling or inflammation, and the cause of the neuropathic pain remains unknown.

**Table 3** AEs and SAEs for phase 2 patients, including relatedness of AEs and SAEs to the various interventions and AEs and SAEs by site

AEs possibly, probably, or definitely related to surgery, device, HSSCs, or IM drug treatment	No. of participants affected	No. of events
<b>Blood disorders</b>	1	2
<b>Gastrointestinal disorders</b>		
Diarrhea	2	3
Nausea/vomiting	3	6
Constipation	5	6
Duodenitis	1	1
Other	2	2
<b>Infections</b>		
UTI	2	3
Pneumonia	3	3
Other	3	3
<b>Injury or procedural complications</b>		
Incision pain	12	19
Nausea secondary to surgery	1	2
Fall	1	2
Other	3	3
<b>Disorders of metabolism and nutrition</b>		
Acute diabetes mellitus	2	2
Hyperglycemia	3	3
Abnormal liver function tests	2	2
Other	2	2
<b>Musculoskeletal disorders</b>		
Muscle weakness	2	4
Muscle pain/ache	3	4
Neck pain/stiffness	7	12
Other	3	3
<b>Nervous system disorders</b>		
Headache	2	2
Hypoesthesia	6	11
Neuralgia	3	8
Paresthesia	4	7
Spinal cord edema	1	1
Myoclonus	1	2
Tremor	2	2
Other	1	1
<b>Psychiatric disorders</b>		
Insomnia	2	2
Hallucination	1	1

Continued

**Table 3** Continued

AEs possibly, probably, or definitely related to surgery, device, HSSCs, or IM drug treatment	No. of participants affected	No. of events	
<b>Renal/urinary disorders</b>			
Urinary incontinence	1	1	
Other	2	3	
<b>Respiratory disorders</b>			
Pleural effusion	1	1	
<b>Skin disorders</b>			
Erythema	1	1	
Other	2	2	
<b>Vascular disorders</b>			
DVT	2	3	
Other	1	1	
<b>Other</b>			
Crepitus	1	1	
Fatigue	1	1	
Ventricular tachycardia (10 beats)	1	1	
<b>AEs and SAEs by site</b>			
<b>Site</b>	<b>No. of AEs</b>	<b>No. of SAEs</b>	
1	50	1	
2	48	3	
3	41	0	
<b>AEs related to intervention, total no. (%)<sup>a</sup></b>			
<b>Intervention</b>	<b>Possibly</b>	<b>Probably</b>	<b>Definitely</b>
Surgery	41 (29)	18 (13)	43 (31)
Device	6 (4)	3 (2)	0 (0)
HSSCs	10 (7)	4 (3)	0 (0)
IM drug	27 (19)	9 (7)	4 (3)

Abbreviations: AE = adverse event; DVT = deep vein thrombosis; HSSC = human spinal cord-derived neural stem cell; SAE = serious adverse event; UTI = urinary tract infection.

<sup>a</sup> Percentages do not add up to 100 because AEs may be related to some degree to more than a single variable.

There is speculation that his history of autoimmune disorders (transverse myelitis and ulcerative colitis) may have predisposed him to an inflammatory reaction within the spinal cord. Another possibility, supported by the delay in onset of symptoms after transplantation, is the proliferation of the transplanted cells, causing irritation of the spinothalamic tracts.

**Analysis of outcomes.** The primary outcome measure for the phase 2 trial was safety, which is addressed in the discussion of AEs above. In addition, we analyzed outcome measures to generate estimates of



slopes of decline as markers of disease progression. Since we did not include a control group in this trial, we compared these slopes of decline to those from 3 historical control groups (see the methods section). It is important to note that, compared to the trial group, the ceftriaxone and ProACT control groups were older and had longer disease duration at entry (table 4). The Emory controls were, by design, matched to the trial participants in this regard. Also note that this trial was neither designed nor powered to assess efficacy, so these statistical comparisons were done as an indication of safety.

Linear mixed models analysis showed no differences between either the combined phase 1 and 2 (n = 24) or the phase 2 only group (n = 15) vs the 3 control groups when looking at slopes of progression for ALSFRS-R, FVC % predicted, and grip strength out to 270 days postsurgery. Thus, the disease progression of trial patients as a group was no worse than that in the control groups, further supporting our conclusion that intraspinal HSSC transplantation is safe. An example of the analysis is provided in table 5, where the trial groups are compared to the ceftriaxone placebo dataset at 270 days after intervention. Data tables that include comparisons of the trial groups to the other control groups at 270 days postintervention are included in tables e-2 to e-4.

Not unexpectedly, there was significant variability among the participants in the slopes of decline for each of the measures of progression. This variability is demonstrated graphically in the figure, which, assuming linearity, shows the slopes for each patient as well as the mean slope for the entire group. Thus, to investigate whether individual participants, rather than all participants pooled as a single group, may have responded differently to the intervention, we calculated confidence limits around the slopes of decline recorded from the 3 control datasets, and asked, patient by patient, whether the slope of decline

fell below, within, or above the confidence limits of the control groups. The confidence limits for mean slopes of decline for the control datasets are shown in table e-5, and the categorization of participants as compared to the control datasets is shown in table e-6. In general, for each of the outcome measures, there were more participants who fell either within or above the upper confidence limit than below the lower confidence limit, supporting the conclusion that the course of disease was not negatively affected by the intervention.

**DISCUSSION** We completed 2 open-label trials testing the safety of intraspinal transplantation of spinal cord–derived neural stem cells in 30 people with ALS. Our phase 1 data<sup>5,6</sup> demonstrated the safety of transplanting a single concentration of HSSCs per injection. The phase 2 trial tested the safety of escalating doses of stem cells delivered in a combination of increasing numbers of cells per injection, numbers of injections, and numbers of procedures. The number of surgical centers was also successfully expanded from a single center to 3, showing that HSSC transplantation will be feasible outside of a single institution for future clinical trial purposes and for widespread application if it is found to be efficacious. This report focuses on the safety and tolerability of these added variables. In addition, in a further effort to evaluate safety, we analyzed measures of disease progression from 24 trial participants, 9 from phase 1 and all 15 from phase 2, comparing their slopes of decline to historical datasets.

Of the 18 procedures performed in 15 participants in phase 2, there were 2 patients who incurred life-altering complications from the treatment—one with postoperative spinal cord swelling that caused pain, sensory loss, and paraparesis, and a second who developed a delayed-onset central pain syndrome requiring

**Table 4** Demographic characteristics of trial participants and control groups

Group (n)	Age, y, mean (SD)	Duration, mean (SD)	Male, %
Phase 1 + 2 (24)	48.7 (10.0)	25.3 (27.2)	79.2
Phase 2 (15)	48.1 (10.4)	15.5 (5.5)	80.0
EU 1 + 2 (32)	49.0 (8.4) <sup>a</sup>	23.5 (19.9) <sup>a</sup>	71.0
EU 2 (19)	48.5 (8.4) <sup>a</sup>	17.6 (7.0) <sup>a</sup>	73.7
CEF placebo (172)	54.8 (10.3) <sup>b</sup>	18.6 (8.0) <sup>c</sup>	50.0
ProACT (552)	55.5 (11.7) <sup>d</sup>	20.6 (11.0) <sup>e</sup>	64.0

Abbreviations: CEF = ceftriaxone trial placebo dataset; EU = Emory University ALS-matched dataset; EU 1 + 2 includes controls for combined phase 1 and 2; EU 2 includes controls for phase 2 only; ProACT = ProACT dataset of control groups.

<sup>a</sup>p = not significant comparing EU 1 + 2 to phase 1 + 2, and EU 2 to phase 2.

<sup>b</sup>p = 0.0065 comparing CEF to phase 1 + 2; p = 0.015 comparing CEF to phase 2.

<sup>c</sup>p = 0.24 comparing CEF to phase 1 + 2; p = 0.14 comparing CEF to phase 2.

<sup>d</sup>p = 0.020 comparing ProACT to phase 1 + 2; p = 0.0157 comparing ProACT to phase 2.

<sup>e</sup>p = 0.55 comparing ProACT to phase 1 + 2; p < 0.0001 comparing ProACT to phase 2.

**Table 5** Parameter estimates from mixed models analysis of change in outcomes over time, study participants vs CEF placebo group

Comparison Parameter	Phase 1 + 2 vs CEF		Phase 2 vs CEF	
	Estimate (SE)	p Value	Estimate (SE)	p Value
<b>ALSFRS-R</b>				
Intercept	36.40 (0.98)	—	37.10 (1.19)	—
Control <sup>a</sup>	-0.10 (0.92)	0.9104	-0.61 (1.16)	0.6007
Time <sup>b</sup>	-0.037 (0.004)	<0.0001	-0.038 (0.004)	<0.0001
Control × time <sup>c</sup>	—	—	—	—
<b>FVC % predicted</b>				
Intercept	88.38 (2.82)	—	88.09 (3.57)	—
Control <sup>a</sup>	-3.33 (2.68)	0.2161	-2.80 (3.46)	0.4200
Time <sup>b</sup>	-0.064 (0.013)	<0.0001	-0.066 (0.013)	<0.0001
Control × time <sup>c</sup>	—	—	—	—
<b>Maximum grip strength</b>				
Intercept	51.21 (3.71)	—	43.98 (4.44)	—
Control <sup>a</sup>	-9.82 (3.47)	0.0052	-1.65 (4.27)	0.6992
Time <sup>b</sup>	-0.048 (0.017)	0.0066	-0.055 (0.018)	0.0020
Control × time <sup>c</sup>	—	—	—	—

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CEF = ceftriaxone trial placebo dataset; FVC = forced vital capacity.

Only observations up to 270 days postintervention (date of surgery for the trial group and initiation of treatment for the CEF group) are used. The cells with an em-dash (—) indicate that a model with the term was evaluated, it was not statistically significant, and thus the estimates and p values from the model without the term are presented.

<sup>a</sup>Control indicates the difference in outcome value at time of intervention for the control group relative to the study group.

<sup>b</sup>Time indicates the rate of outcome value change per day since (first) intervention.

<sup>c</sup>Control × time indicates whether the control group changes at a different rate than trial participants.

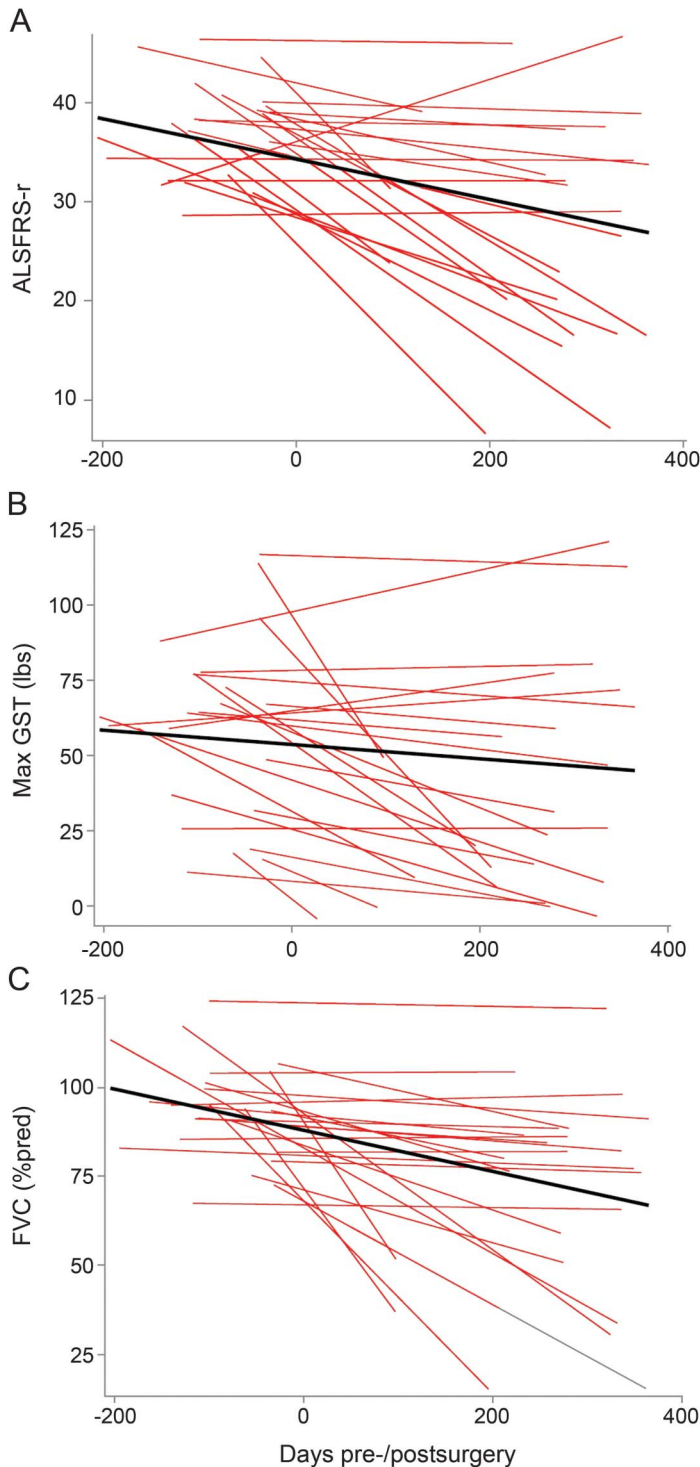
ongoing interventions for pain management. The cause or causes of these complications are debatable, with surgical trauma and inflammatory reaction to the transplanted cells remaining possibilities. Both of these patients received the highest dose of cells into the cervical cord (20 injections, 400,000 cells/injection), which suggests caution for the choice of dosing for the next phase of the trial. However, 4 other patients received this same dose of cells without apparent complications, and 10 other patients received 20 cervical injections with various concentrations of cells without complications. The 3 patients in cohort E tolerated successive procedures, although the patient with chronic pain was in this group.

In the development of a new therapeutic intervention, safety is defined relative to the severity of the disease. The level of acceptable risk for treating patients with ALS, whereby the prognosis is poor and disease-modifying interventions are limited, is arguably higher than that for more benign disorders. Here, we have shown that, with a few exceptions, patients with ALS can tolerate up to 20 injections with 400,000 cells per injection into the lumbar and cervical spinal cord, including 2 successive procedures totaling 40 injections and 16 million cells. Thus, we conclude that

the injection procedure, as well as the introduction of high doses of HSSCs into the spinal cord, is relatively safe. Certainly, we base these conclusions on the limited number of participants treated in each group. In addition, we succeeded in moving this complex procedure from a single surgical center to 3 centers, demonstrating that this therapeutic approach is amenable to scaling up for the treatment of larger numbers of participants in a multicenter trial of efficacy.

Our statistical analysis focused on comparing the trial participants with observational datasets selected from the placebo arms of previous ALS clinical trials and from the large patient database housed at Emory University. The ceftriaxone and ProACT datasets were used because they represent previous ALS trial populations and are publically available. The Emory dataset was generated by matching individual trial participants with patients with similar disease courses based on disease duration, sex, and age at onset of symptoms, which created a “control” group likely more comparable to the trial group, although certainly more limited in numbers than the other comparison groups. Slopes of progression for ALSFRS-R, FVC % predicted, and grip strength did not differ

**Figure** Linear regression lines of individual (red lines) and average fitted slopes (black lines) of decline for the 3 outcome measures



(A) ALSFRS-R, (B) maximum GST (lb), (C) FVC (% predicted). Linearity is assumed from pre-intervention to 12 months postintervention to illustrate wide variability of disease progression among the phase 1 and 2 participants. ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; FVC = forced vital capacity; GST = grip strength.

between the trial participants and any of the control groups. This was true whether combining the phase 1 and 2 participants, or analyzing the phase 2 participants alone. From the standpoint of safety, this is a positive

outcome since it was our overriding goal to assess whether surgical implantation of HSSCs accelerates disease progression or is otherwise harmful to ALS participants. We acknowledge, however, the limitations of using any historical control group in assessing either safety or efficacy given the lack of contemporaneous matching and variations in clinical care that may lead to changes in the natural history of disease.<sup>17,18</sup> This is particularly relevant when studying a disease with such a variable course of progression.

Another approach for evaluating safety would include comparing slopes of progression presurgery to those postsurgery. However, this study was not designed to test efficacy and we had too few data points for each patient to establish an effective lead-in slope with which to compare the postsurgery slope.

It is difficult to assess efficacy in such a small treatment trial, especially one with no randomized placebo or observational comparison group.<sup>18</sup> In addition, each treatment cohort included only 3 participants, further limiting our ability to make any conclusive statements about therapeutic efficacy.<sup>19</sup> The clinical progression in ALS is highly variable, and the biological factors accounting for this variability are unknown. Thus, we must be cautious about ascribing cause and effect for differences in disease course between individual participants. We addressed individual variability by calculating confidence limits around the slopes of decline for the patients in the historical datasets. The finding that the slopes of decline for our patients were similar to those from the historical control groups is a further indication that this procedure does not negatively affect the course of disease.

This study was not designed, nor was it large enough, to determine efficacy of slowing or stopping the progression of ALS. The design of such an efficacy trial will be a challenge given the ethics surrounding the inclusion of a true placebo group: participants receiving injections into the spinal cord with saline only. Alternatives include a blinded surgical control group that is not exposed to cord penetration, or a randomization scheme that includes a concurrent untreated observational group. Another approach could be to use individual participants as their own controls, comparing the slopes of decline before and after treatment. For this model, the choice of participants would be predicated largely on the presence of a moderate slope of decline measured during a 3- to 6-month lead-in period, with both rapid and slow progressors eliminated, allowing the use of predictive models to test whether a slope of decline has been positively affected by the treatment. Such predictive models have already been developed<sup>20</sup> and are certain to be used to design and power future ALS therapeutic trials.



## AUTHOR CONTRIBUTIONS

J.D.G., E.L.F.: protocol development, experimental procedures, patient care, data analysis, original and final draft of manuscript. V.S.H.: protocol development, experimental procedures, statistical analysis, original and final draft of manuscript. C.F., M.C., N.A., S.A.G.: experimental procedures, patient care, original and final draft of manuscript. S.B.R.: protocol development, experimental procedures, final draft of manuscript. N.M.B., J.R., P.G.P., L.F.B.: surgical procedures and final editing of manuscript. T.F.: data collection and analysis. K.J., T.H.: cell production and characterization and final editing of manuscript. M.P., J.B., J.D.: patient care, data collection, final editing of manuscript.

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