Intraspinal Stem Cell Transplantation in Amyotrophic Lateral Sclerosis: A Phase I Trial, Cervical Microinjection, and Final Surgical Safety Outcomes

Jonathan Riley, MD*
Jonathan Glass, MD‡
Eva L. Feldman, MD, PhD§
Meraida Polak, BSN, RN‡
Jane Bordeau, RN‡
Thais Federici, PhD*
Karl Johe, PhD¶
Nicholas M. Boulis, MD*

*Department of Neurosurgery, Emory University, Atlanta, Georgia; ‡Department of Neurology, Emory University, Atlanta, Georgia; §Department of Neurology, University of Michigan, Ann Arbor, Michigan; ¶Neuralstem, Inc, Rockville, Maryland

Correspondence:
Nicholas Boulis, MD,
Emory University,
Department of Neurosurgery,
1365-B Clifton Rd NE, Suite B6200
Atlanta, GA 30322.
E-mail: nboulis@emory.edu

Received, May 25, 2013.
Accepted, August 30, 2013.
Published Online, September 5, 2013.

WHAT IS THIS BOX?
A QR Code is a matrix barcode readable by QR scanners, mobile phones with cameras, and smartphones. **The QR Code above links to Supplemental Digital Content from this article.**

BACKGROUND: The first US Food and Drug Administration approved clinical trial for a stem cell-based treatment of amyotrophic lateral sclerosis has now been completed.
OBJECTIVE: Primary aims assessed the safety of a direct microinjection-based technique and the toxicity of neural stem cell transplantation to the ventral horn of the cervical and thoracolumbar spinal cord. Results from thoracolumbar-only microinjection groups have been previously published. Cervical and cervical plus thoracolumbar microinjection group perioperative morbidity results are presented.
METHODS: Eighteen microinjection procedures (n = 12 thoracolumbar [T10/11], n = 6 cervical [C3-5]) delivered NSI-566RSC (Neuralstem, Inc), a human neural stem cell, to 15 patients in 5 cohorts. Each injection series comprised 5 injections of 10 μL at 4-mm intervals. The patients in group A (n = 6) were nonambulatory and received unilateral (n = 3) or bilateral (n = 3) thoracolumbar microinjections. The patients in groups B to E were ambulatory and received either unilateral (group B, n = 3) or bilateral (group C, n = 3) thoracolumbar microinjection. Group D and E patients received unilateral cervical (group D, n = 3) or cervical plus bilateral thoracolumbar microinjection (group E, n = 3).
RESULTS: Unilateral cervical (group D, n = 3) and cervical plus thoracolumbar (group E, n = 3) microinjections to the ventral horn have been completed in ambulatory patients. One patient developed a postoperative kyphotic deformity prompting completion of a laminoplasty in subsequent patients. Another required reoperation for wound dehiscence and infection. The solitary patient with bulbar amyotrophic lateral sclerosis required perioperative reintubation.
CONCLUSION: Delivery of a cellular payload to the cervical or thoracolumbar spinal cord was well tolerated by the spinal cord in this vulnerable population. This encouraging finding supports consideration of this delivery approach for neurodegenerative, oncologic, and traumatic spinal cord afflictions.

KEY WORDS: Amyotrophic lateral sclerosis, Cell therapy, Intraspinal microinjection, Spinal cord injection, Stem cell

Neurosurgery 74:77–87, 2014 DOI: 10.1227/NEU.0000000000000156 www.neurosurgery-online.com

ABBREVIATIONS: ALS, amyotrophic lateral sclerosis; **ALSFRS-R,** revised Amyotrophic Lateral Sclerosis Functional Rating Scale; **FDA,** US Food and Drug Administration; **MEP,** motor evoked potential; **POD,** postoperative day; **SSEP,** somatosensory evoked potential

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.neurosurgery-online.com).

The first US Food and Drug Administration (FDA)-approved trial to treat amyotrophic lateral sclerosis (ALS) with a cell-based therapeutic and a direct microinjection-mediated spinal cord delivery approach has concluded enrollment. The biological payload, NSI-566RSC, is a human fetal spinal cord-derived neural stem cell line that has demonstrated safety and efficacy in preclinical small-animal models of ALS.[1,2] Fifteen patients in 5 cohorts have received 18 thoracolumbar and/or cervical microinjection

procedures delivering NSI-566RSC to the ventral horn with either unilateral or bilateral cell injections. The first patient received treatment in January 2010, and the final microinjection procedure took place in August 2012. All procedures were completed at Emory University Hospital in Atlanta, Georgia.

Cervical Microinjection Outcomes

We have recently reviewed the multiple completed and ongoing domestic and international trials delivering cell-based therapies to the spinal cord.[3-5] These trials included the treatment of ALS or alternative afflictions (eg, spinal cord injury, spinal muscular atrophy). A broad spectrum of delivery approaches (eg, intravascular, intrathecal, direct microinjection), cell-based therapies (eg, autologous vs allogeneic), and strategies for immunosuppression (eg, none, single agent, or multiagent) have been attempted. The design of the current trial was influenced by each of these previous experiences. First, we used a patient-stabilized microinjection platform that was tested in preclinical large animal studies.[4,6-8] Second, an allogeneic cellular graft with supportive preclinical efficacy data was used as the payload.[1] Third, a multiagent immunosuppressant regimen was used.[9] Finally, we used a risk escalation paradigm of trial design. In this approach, thoracolumbar microinjections were performed before attempts at cervical intervention, and nonambulatory patients received grafts before ambulatory patients. Progression to cervical microinjection cohorts required an interim analysis by the FDA with demonstration of safety in nonambulatory and ambulatory thoracolumbar microinjection patients.

Interim results for safety[10] and functional[11] outcomes were previously published for thoracolumbar-only microinjection patients. These results support the safety of serial bilateral thoracolumbar microinjection in ambulatory patients and do not show acceleration of disease related to the injection procedure. Additionally, there was an early finding of clinical improvement in 1 patient. This article presents the perioperative morbidity data for ambulatory patients who underwent serial unilateral cervical and cervical + bilateral thoracolumbar microinjection. This represents 6 of the 15 enrolled patients, comprising 2 of the 5 cohorts. Finally, the experiences gained through both our extensive preclinical work and through completion of this phase I trial are used as a foundation to discuss relevant considerations for future efforts to translate cell-based therapies to the human spinal cord.

PATIENTS AND METHODS

Trial Design

The trial design has been published[12] and is briefly reviewed. This open-label phase I safety trial was based on a "risk escalation" paradigm in which successive patient cohorts have both improved functional capacity and are subjected to a progressive element of risk to neurological function. There were 5 patient cohorts. Progression between groups (A to E) and ultimate trial completion required interval demonstration of procedural safety. Thoracolumbar-only microinjections were completed in groups A to C. Group A (n = 6) patients received unilateral or bilateral microinjection (n = 3 each) and were nonambulatory. Group B and C (n = 3 each) patients were ambulatory and received unilateral (B) or bilateral (C) microinjection. Interim results for the primary (safety) outcome[10] and secondary (functional) outcomes for groups A to C have been published.[11] Group D (n = 3) and Group E (n = 3) comprised ambulatory patients, who received unilateral cervical or unilateral cervical + bilateral thoracolumbar microinjection, respectively. Group D and E safety outcomes are reported here. Secondary trial end points have assessed disease outcome-related measures and will be reported separately.

Cell Suspension

NSI-566RSC (Neuralstem, Inc; Rockville, MD) is a human fetal neural stem cell line obtained from a fetal spinal cord of approximately 8 weeks gestational age. A tissue area corresponding to the lower cervical/upper thoracic cord was obtained and separated from associated meninges and dorsal root ganglia. Neural stem cells were isolated and propagated. This expanded cell line has been validated through both in vitro preclinical studies and in the superoxide dismutase 1 familial ALS small-animal model. It has been demonstrated to prolong motor neuron survival and improve overall superoxide dismutase 1 small-animal survival[1] while expressing a largely GABAergic phenotype.[13] Completion of in vitro and small-animal safety studies has demonstrated a lack of tumorigenicity; microarray and immunohistochemical analyses have shown low levels of human leukocyte antigen expression and a lack of immunoreactivity to ABO antigens.[11] Further, the cell line tested negative for several disease-causing agents, including bacteria, mycoplasma, and multiple known viral pathogens.[11] The cell suspension is prepared to a final concentration of 1×10^4 cells/μL at 2 to 8°C in a Cryovial with a volume of 0.5 to 1 mL. Cellular graft preparation upheld appropriate FDA and National Institutes of Health guidelines. For full details regarding NSI-566RSC cell line characteristics, immunogenic profile, preclinical evaluation, or adherence to National Institutes of Health and FDA guidelines, please see the previous full descriptions.[10,11]

Enrollment Criteria

Enrolled patients satisfied the El Escorial criteria for a diagnosis of ALS, ruling out other major categories of neurological disease. They were deemed safe candidates for surgical intervention. Further, enrolled patients underwent a rigorous institutional review board-reviewed process emphasizing the source of the implanted cells and that participation did not carry an expectation of prolonged survival. A detailed set of enrollment inclusion and exclusion criteria may be found at the following website: http://www.clinicaltrials.gov/ct2/show/NCT01348451. Further details regarding patient selection, recruitment, and the informed consent process may be found in reference.[11] Table 1 provides basic demographic and functional data for patients from groups D and E.

Immunosuppression

Enrolled patients are planned to receive lifelong multiagent immunosuppression, consistent with the current standard of care for solid organ transplantation.[9] Development of a postoperative infection or an attributable immunosuppressant-related toxicity constitute the discontinuation criteria. For full details regarding the immunosuppressant regimen, please see either the interim primary[10] or secondary outcome trial results[11] or a separately published description of the study protocol.[12] An abbreviated description of the regimen is presented. Methylprednisolone 125 mg IV (Solumedrol, Pfizer, Inc, New York City, New York) was administered at

TABLE 1. Cervical Microinjection Demographic Data

Group	Trial Patient NO.	Operation No.	Age, Sex	Disease Duration	Surgery Date[a]	Length of Stay, Days
D						
D1	13	13	50, M	3 y, 1 mo	11/18/2011	5
D2	14[b]	14	54, F	2 y, 9 mo	2/29/2012	5
D3	15	15	35, F	2 y, 8 mo	4/18/2012	5
E[c]						
E1	10	16	50, M	8 y, 7 mo	6/13/2012	4
E2	12	17	56, M	2 y, 11 mo	7/20/2012	4
E3	11	18	40, M	2 y, 2 mo	8/21/2012	6

[a]Group E patients had a previous lumbar microinjection surgery date. The date provided represents the cervical microinjection date.
[b]Patient 14 is documented to have bulbar predominant amyotrophic lateral sclerosis.
[c]Group E patients previously underwent bilateral thoracolumbar microinjection as participants in group C. The trial patient number reflects the initial numbering from group C.

2 hours before incision and over a subsequent 28-day oral taper, with a dose reduction each week (60 mg, 40 mg, 20 mg, 10 mg). Basiliximab 20 mg IV (Simulect, Novartis, East Hanover, New Jersey) was administered twice, first at the time of dural opening and again on postoperative day 4 (POD4). The 2 remaining agents continue to be administered on an ongoing basis. Tacrolimus 0.1 mg/kg per day (Prograf Fujisawa Healthcare, Inc, Deerfield, Illinois) is given with twice daily dosing, beginning on POD1. Mycophenolate mofetil 500 mg by mouth (Cellcept, Roche Laboratories Inc, Nutley, New Jersey) is first given orally twice per day on POD 1 and is titrated to a final dose of 1 g given twice daily by POD14.

Patient Positioning and Neuromonitoring

Padded chest rolls and a Mayfield head holder were used for the cervical microinjection approach, as shown in Figure 1A. The patient's head was placed in mild capital flexion, and tape was placed on the shoulders to improve fluoroscopic visualization during the operative exposure. Figure 1B demonstrates generous hair clipping to the level of the external occipital protuberance to accommodate the rostral microinjection platform posts. Neuromonitoring was used to allow assessment of somatosensory evoked potentials (SSEPs) during microinjection. The trial protocol required termination of injection if SSEP values fell to 50% of baseline without recovery within a 30-minute period. Surgical site preparation included the use of Hibiclens (4% chlorhexidine gluconate) followed by Betadine scrub and paint.

Surgical Technique and Microinjection

Fluoroscopy was used to obtain positive confirmation of the appropriate levels for both thoracolumbar (T10/11) and cervical (C3-5) exposure. A standard midline posterior approach was used with completion of a T10/11 or C3-5 laminectomy. Secondary to the development of a progressive postoperative kyphosis in trial patient 13 (group D, patient 1), subsequent microinjection patients (16-18, respectively, trial patients 10, 12, and 11) received a cervical laminoplasty. After completion of the bony decompression, the microinjection platform base, shown in Figure 2A-C, was attached to a custom self-retaining retractor system. Figure 2A demonstrates the microinjection platform base. Figure 2B demonstrates incorporation of an

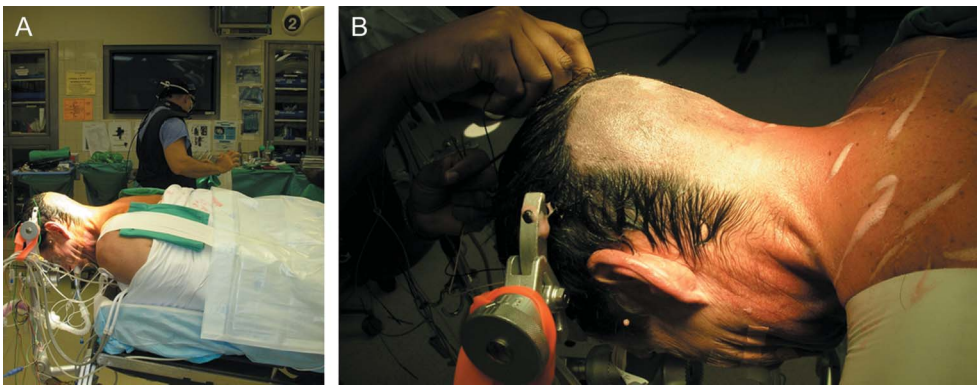

FIGURE 1. *Patient positioning for cervical microinjection.* **A**, *the patient was positioned prone. The chest was supported by gel rolls and the head immobilized with the use of a Mayfield head holder. Mild capital flexion is used. The shoulders were gently taped to optimize intraoperative fluoroscopic evaluation of the operative levels. Neuromonitoring was used.* **B**, *a close-up view demonstrates wide hair clipping to the level of the external occipital protuberance for rostral post placement.*

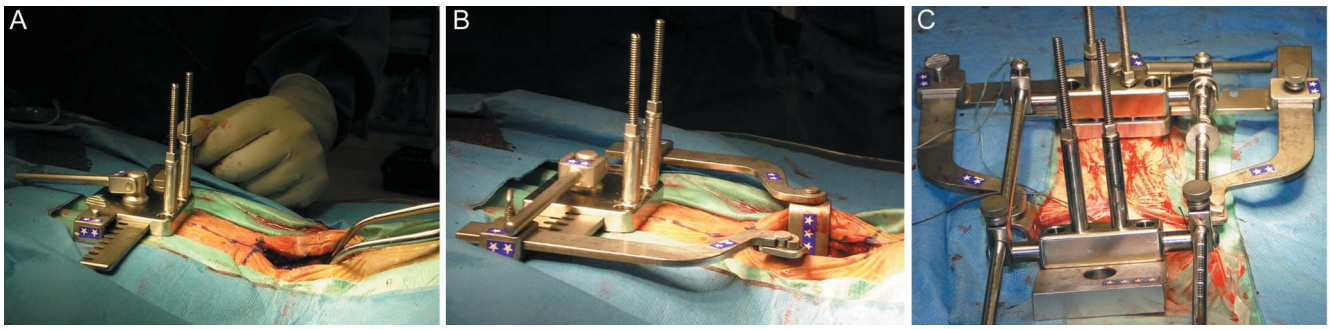

FIGURE 2. *Cervical microinjection device application. A standard midline posterior approach was used. In this series, exposure of the C2 to C6 vertebrae was obtained with care taken to prevent injury to the adjacent level facets at the rostral (C2/3) and caudal (C5/6) extent of the exposure. Rostral fixation of the microinjection platform was to the calvarium.* **A**, *percutaneous posts were placed. Caudal posts were placed at approximately the T1 level with purchase into the lamina.* **B**, *platform retractors have now been added, maintaining the paraspinal musculature out of the field during microinjection.* **C**, *the rostral retractor system has been added, partially stabilized by anchoring in the paraspinal musculature with attachment to the occiput. Additionally, the rail system that accommodates the microinjector and floating cannula is demonstrated.*

integrated retractor system. In Figure 2C, the rostral posts, rostral platform base, and rail system were added. At this point, dural opening and tack-up were completed. Images of the microinjector and floating cannula have been previously demonstrated.[6,10] Five sequential unilateral injections were completed (rostrocaudal spacing 4 mm) with the precalibrated MINI-PD microINJECTOR 8 pump (Tritech Research, Inc, Los Angeles, California). Injection targeting to the ventral horn was anatomically based and entails penetration 1 to 2 mm medial to the dorsal rootlet entry zone, to a depth of 4 to 5 mm. The final depth was based upon preoperative imaging. The anatomic targeting approach was discussed in detail in the interim safety outcomes article[10] and was based on significant preclinical experience with microinjections in swine. The cannula was introduced into the spinal cord in rigid confirmation. Appropriate depth was indicated when the microinjection needle tip flange was flush with the spinal cord. At this point, the rigid outer cannula was drawn back. This allowed the needle tip and connected Silastic tubing to "float" with ventilation-associated cord excursion and cardioballistic-associated spinal cord pulsation. The injection process was recorded with the operative microscope. This allowed precise postsurgical graft site localization. Reflux was minimized by observance of a 60-s pause following injection completion before cannula withdrawal. An online supplement to the interim safety trial results[10] demonstrates a video of the microinjection process, and may be found here: http://links.lww.com/NEU/A454. In this article, a new video is provided that highlights the increased degree of spinal cord excursion during cervical microinjection (see **Video 1, Supplemental Digital Content 1**, http://links.lww.com/NEU/A588, which details the cervical microinjection process and cervical cord excursion). This was largely attributable to patient movement during ventilation. A video demonstrating the entire surgical approach and microinjection procedure may be found in reference.[14] A description of the evolutionary development of the microinjection platform and cannula hardware may be found here.[6] Dural closure was completed with a running 4-0 Nurolon suture. Fascia was closed with 2-0 Prolene, subcutaneous layer closed with inverted interrupted 2-0 Vicryl, and skin with running 2-0 Ethilon.

Postoperative Outcome Measures

The primary trial objective has been to assess the safety of cellular delivery to the human spinal cord. This was assessed by collection of adverse events that may be considered as either procedurally related or graft-related. Interim primary outcome measure results may be found here.[10] The former are attributable to the surgical intervention (eg, postoperative kyphosis, surgical site infection), whereas the latter are attributable to the presence of an indwelling cellular graft within the spinal cord (eg, graft rejection, cell proliferation). Secondary outcome measures include quantitative and semiquantitative measures of ALS progression. Those reported herein include percent predicted vital capacity and revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) scores. Interim secondary outcome results may be found here.[11] In addition to adverse event and outcome measure collection, postoperative magnetic resonance imaging (MRI) (t = 1, 6, 12 months) was obtained to evaluate possible tumorigenesis or immune activation. Finally, histological analysis has been completed on deceased trial participants to evaluate for viable graft. Imaging and histological data will be separately presented.

RESULTS

Patient Demographic Information

A total of 6 (4 male, 2 female) patients underwent unilateral cervical microinjection. Each received a series of 5 unilateral injections. Patient age ranged from 35 to 56. Half (n = 3) of these patients (group E) had undergone previous bilateral lumbar microinjection with a series of 5 injections per side (n = 10 total thoracolumbar injections). A summary of patient demographics may be found in Table 1. Postoperative follow-up for groups D and E is ongoing. Trial patient 14 (group D patient 2) was diagnosed with bulbar-predominant ALS. Two patients in this trial are female (group D patients 2, 3; Trial patients 14, 15). All group D and E patients have thus far received a minimum of 7 months of follow-up from cervical microinjection.

Intraoperative Findings

Table 2 summarizes the intraoperative findings for the patients receiving cervical microinjection. SSEPs and motor evoked

potentials (MEPs) were recorded on all patients. Clinical decisions were not made based on MEP values, because these have not been previously documented in ALS patients. No changes in SSEPs or MEPs were noted throughout any of the cervical microinjection procedures. A total of 30 spinal cord penetrations and injections were completed in these 6 patients. Two episodes of mild venous tract hemorrhage were noted following injection needle removal (6.7%; 2 in 30 injections), with minimal oozing from a cord penetration site. This was well controlled with temporary application of Gelfoam and micropattie placement. No bleeding persisted following Gelfoam and micropattie placement. Neither intraoperative spinal cord swelling when seen under direct visualization nor postoperative morphological cord changes on MRI imaging obtained at 3 and 6 months were observed. The operative time for group E (298, 374, 313 minutes) was longer than observed for group D (234, 269, 252 minutes). This is attributable to laminoplasty hardware placement, cervical wiring, and the additional care required to maintain the C3-5 posterior elements out of the operative field during microinjection in group E patients.

Postoperative Neurological Findings

The length of postoperative inpatient stay ranged from 4 to 6 days. All patients were nonventilated and ambulatory at preoperative baseline by time of discharge. All patients were extubated in the operating room. One patient (group D, patient 2; trial patient 14) required reintubation and respiratory support. This patient, diagnosed with bulbar predominant ALS, was extubated on POD 2. No patients demonstrated evidence of significant bowel or bladder dysfunction. Figure 3 provides perioperative percent vital capacity (Figure 3A, C) and ALSFRS-R (Figure 3B, D). Preoperative baselines are compared with the first measured perioperative outcomes. Aside from the bulbar ALS patient, who is the only patient receiving cervical microinjection to have died to date ($t = 6$ months postoperatively), no patients demonstrated decrement of the ALSFRS-R or percent vital capacity measures. Further, no patients developed a focal neurological worsening in the perioperative period. Adverse events are summarized in Table 3.

Postoperative Kyphotic Deformity

All trial patients have completed postoperative surveillance imaging up to a minimum time point of 6 months postoperatively. A single patient (group D, patient 1; trial patient 13) has developed a progressive postoperative kyphotic deformity, as shown in Figure 4. Figure 4A demonstrates a preoperative baseline. Figure 4B and C, respectively, demonstrate postoperative months 3 and 6. Figure 4D is a lateral plain radiograph at 6 months. This patient has, to date, elected not to undergo further surgical intervention. Subsequent trial patients (group E, patients 1-3; trial patients 10-12) received a cervical laminoplasty following completion of the microinjection procedure. Figure 5 demonstrates preoperative (Figure 5A) and 3-month postoperative images (Figure 5B,C) in a patient who received cervical laminoplasty hardware.

TABLE 2. Cervical Microinjection Intraoperative Findings[a]

Group	Trial Patient No.	Ambulation Status	Operation No.	Unilateral vs Bilateral	Injection Side	Operative Time, min	Injection Tracts Per Patient	Intraoperative Cord Hemorrhage	Other
D		Ambulatory							
D1	13		13	Unilateral	R	234	5	Not observed	SSEP unchanged
D2	14		14		L	269	5	1 mild tract hemorrhage	SSEP unchanged
D3	15		15		R	252	5	Not observed	SSEP unchanged
E[b]		Ambulatory					5 (10 prior lumbar)		
E1	10		16	Unilateral	L	298	5	1 mild tract hemorrhage	SSEP unchanged
E2	12		17		L	374	5	Not observed	SSEP unchanged
E3	11		18		L	313	5	Not observed	SSEP unchanged

[a]SSEP, somatosensory evoked potential.
[b]Group E patients underwent bilateral lumbar microinjection procedure during group C. Groups E and C represent the same patients.

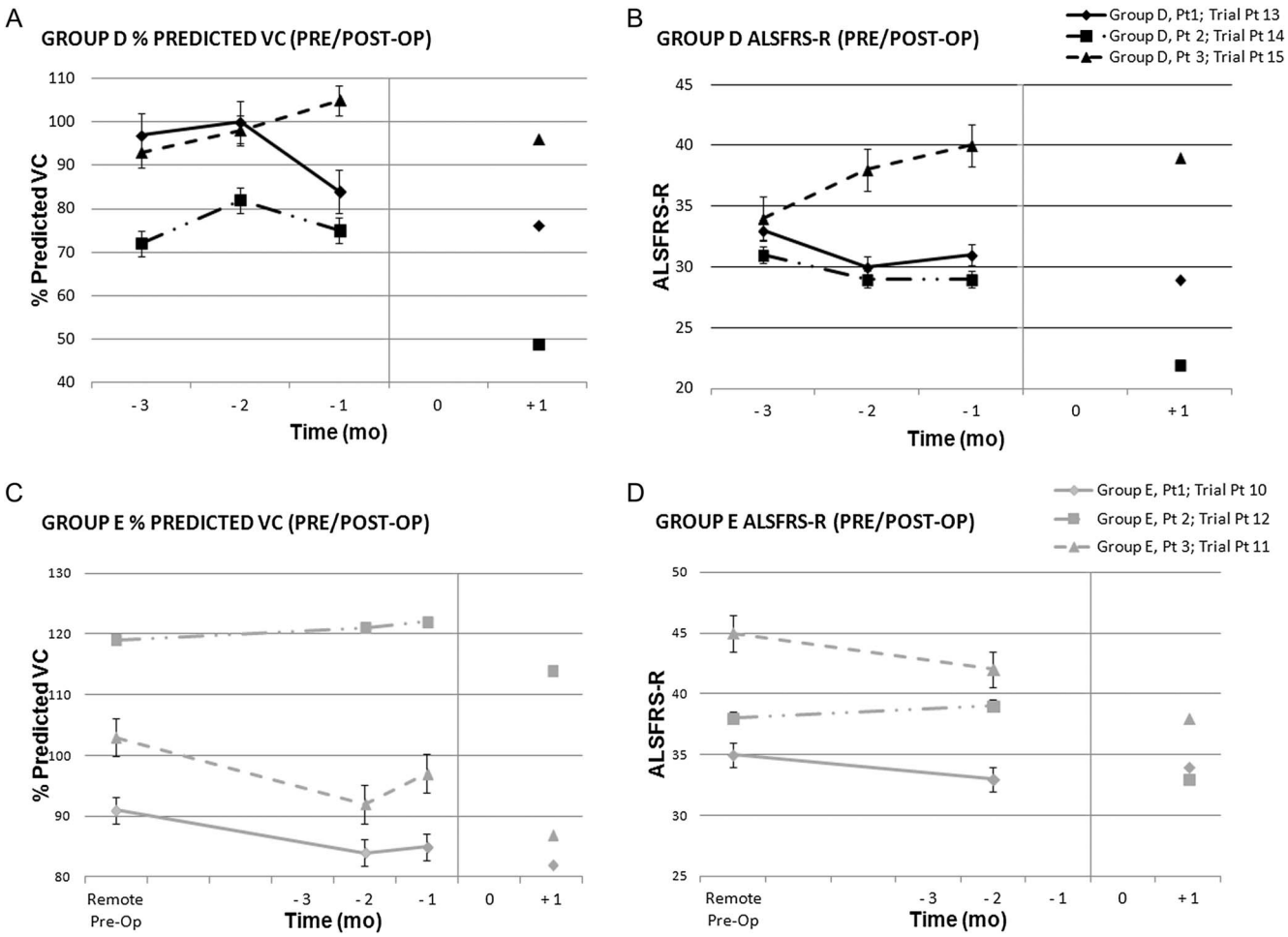

FIGURE 3. *Peri-operative neurological outcomes. Pre- and postoperative results for the percent predicted vital capacity (VC) and ALSFRS-R score are provided. Postoperative scores for all patients are provided at* t *= 1 month postoperatively. Standard error bars are provided.* **A**, **B**, *three months of preoperative data are provided for group D patients. Both the postoperative percent predicted VC and ALSFRS-R score are worsened for group D, patient 2, the patient with a diagnosis of bulbar ALS.* **C**, **D**, *group E patients, who underwent a previous thoracolumbar injection received a remote preoperative assessment at either 5 or 6 months preoperatively. They also received a preoperative VC measurement at 2 and 1 months, preoperatively. A remote preoperative assessment, at 6 or 5 months preoperatively, was obtained for the ALSFRS-R score along with a final preoperative time point at 2 months. ALS, amyotrophic lateral sclerosis; ALSFRS-R, revised Amyotrophic Lateral Sclerosis Functional Rating Scale; Pt, patient.*

Surgical Reintervention

A single group E patient (group E patient 3; trial patient 11) required reoperation for superficial wound dehiscence and infection 2 weeks postoperatively. Of note, this patient had already been on chronic immunosuppression as a previous member of group C. With consultation from the Infectious Disease service, superficial wound irrigation and debridement with operative reclosure was performed. Intraoperative cultures were positive for a polymicrobial infection. The patient received a peripherally inserted central catheter and an outpatient course of intravenous antibiotics. The patient subsequently underwent a second reoperation, wound culture, and hardware removal secondary to the presence of ongoing neck pain. No purulence or evidence of deep infection was noted during the deep wound exploration and hardware removal. Tacrolimus was temporarily discontinued in this patient as a result of temporary acute renal failure that developed after the initiation of intravenous antibiotics.

DISCUSSION

This article presents the perioperative cervical microinjection safety outcomes for an intraspinal microinjection approach designed to deliver a cellular payload to the ventral horn of the ALS spinal cord. When combined with recently published perioperative safety data for delivery to the thoracolumbar spine,[10]

TABLE 3. Perioperative Adverse Events for Microinjection Groups D and E[a]

Adverse Event Category	Group No., Patient No.	Severity[b]	Duration, Days	Comments
Operative				
Wound dehiscence	E, 3	3	45	• Re-Op 1: superficial wound washout • Re-Op 2: Deep Cx and hardware removal
Non-operative				
Pain				
Incisional	E, 1	2	36	
	E, 3	3	64	
	E, 2	2	45	
	D, 1	3	53	
	D, 2	2	10	
	D, 3		53	
Muscle spasm	E, 3	2	69	
	E, 2	2	45	
	D, 1	3	88	• Attributable to progressive kyphosis
Neck pain	E, 3	4	30	
	D, 1	2	88	• Attributable to progressive kyphosis
	D, 3	2	43	
Headache	E, 1	2	1	
	D, 2	3	41	
	D, 2	2	30	
Cervical kyphosis	D, 1	2	Ongoing	
Bowel/bladder				
Urinary retention	E, 3	1	2	
Constipation	E, 1	1	1	
Motor[c]				
Other				
Grinding in neck	D, 1	2	Ongoing	• Attributable to progressive kyphosis
Laryngeal edema	D, 2	4	5	• Required reintubation; Dx bulbar ALS
Shoulder pain	D, 3	2	14	
Nausea/vomiting	E, 2	1	4	
Hiccups	E, 3	2	27	
	E, 2	2	13	
Popliteal vein thrombosis	E, 2	2	50	• Subsequent imaging demonstrated recanalization and clot regression

[a]ALS, amyotrophic lateral sclerosis; Re-Op, reoperation; Cx, culture; Dx, diagnosis; ADLs, activities of daily living; Inc sep, incisional separation; N/V, nausea/vomiting; IVF, IV fluids; TF, tube feeds.

[b]Severity grading: 4 point scales, legend for observed scores shown. *Pain/spasm/headache*: (1) mild—not interfering with function, (2) Mod—pain or medications interfering with function but not ADLs, (3) Severe, (4) Disabling. *Cervical Kyphosis*: 2—moderate. *Grinding in Neck*: 2—moderate. *Dehiscence*: (1) Inc sep <25%; suprafascial dehiscence, (2) Inc sep >25%, (3) Inc sep >25% and possible fascial disruption. *Popliteal Thrombosis*: 2—Deep vein thrombosis or cardiac thrombosis; intervention (eg, anticoagulation, lysis, filter, invasive procedure) not indicated. *N/V/Ileus*: (1) Loss of appetite without change in eating, (2) 2-5 episodes in 24 hours. IVF indicated <24 hours, (3) Inadequate caloric intake. IVF or TF. *Urinary Retention*: 1—Hesitancy or dribbling, but no significant residual urine; retention occurring during the immediate postoperative period. *Constipation*: 1—Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema.

[c]No clinical documentation of worsened motor examination from baseline by discharge.

the primary outcome measure findings from this open-label phase I trial support the safety of a targeted direct microinjection approach to the already compromised and vulnerable ALS spinal cord. Specifically, the ALS spinal cord has shown an ability to tolerate up to 15 microinjections (5 unilateral cervical and 10 bilateral thoracolumbar; 150 μL total volume; 1.5 million delivered cells) without evidence of intraoperative (eg, SSEP decline) or postoperative neurological sequelae. Further, preclinical large-animal data have supported the safety of the maximum number of tested injections, 40 cervical microinjections (20/side) (N.M.B., J.R., unpublished data). When the provided intraoperative videos for thoracolumbar (http://links.lww.com/NEU/A454) and cervical microinjection procedures (see **Video 1, Supplemental Digital Content 1**, http://links.lww.com/NEU/A588, which details the cervical microinjection process and cervical cord excursion) were compared, ventilation-associated spinal cord excursion was observed to be significantly more pronounced in the cervical spinal cord.

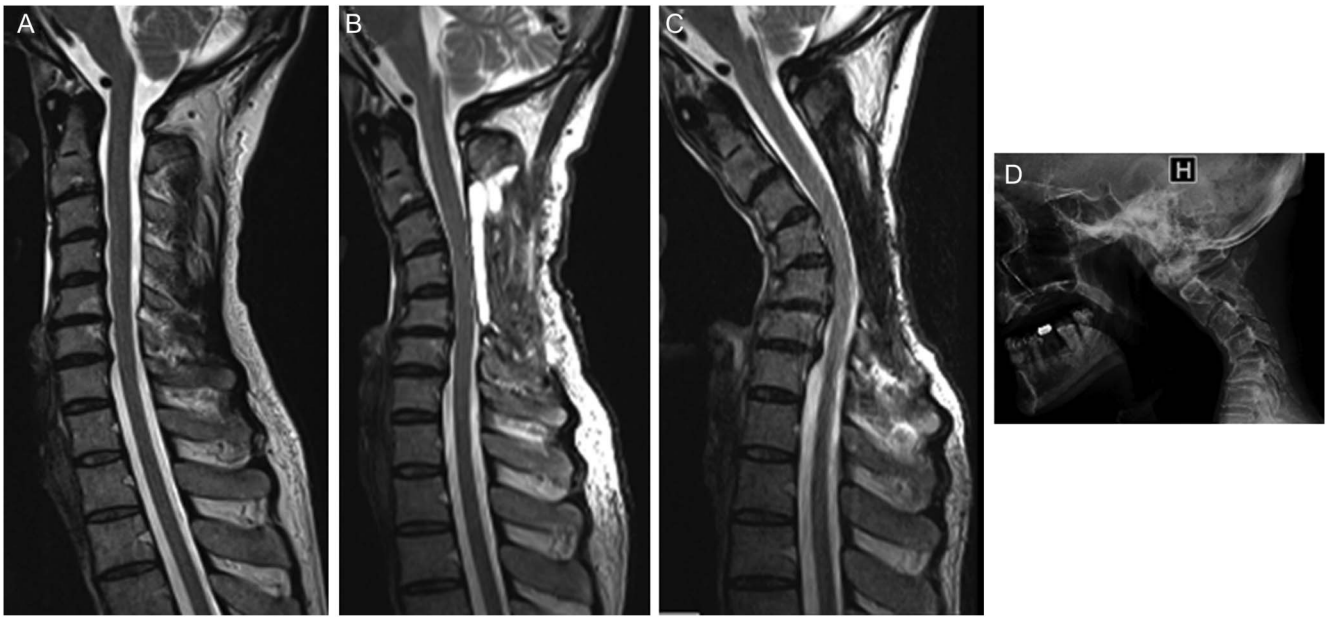

FIGURE 4. *Post-operative kyphosis. Despite care to preserve the facet joints at each level of the dissection, including the rostral and caudal extent of the exposure (C2/3) and (C5/6), the first cervical microinjection patient (trial patient 13) developed a progressive kyphotic deformity. There was no radiographic evidence of myelomalacia or postinjection cord injury.* **A**, *a midsagittal preoperative T2-weighted MRI demonstrates a loss of normal cervical lordosis.* **B**, *an MRI obtained at 3 months postoperatively demonstrates the development of a kyphotic deformity with draping of the spinal cord over cervical vertebrae.* **C** *and* **D**, *respectively, demonstrate a midsagittal T2-weighted MRI and lateral plain radiograph obtained at 6 months postoperatively. These demonstrate of the postsurgical kyphotic deformity. MRI, magnetic resonance imaging.*

Incorporation of the floating cannula, capable of accommodating for this movement, appears crucial to the lack of morbidity observed in human and large-animal cervical spinal cord microinjections. Although the presented thoracolumbar and cervical perioperative safety data are encouraging, several issues require further consideration. Broadly, these include (1) management strategies for observed postoperative kyphotic instability, (2) approaches to mitigate perioperative risk (eg, ALS variants, chronic immunosuppression), (3) methods to further tailor immunosuppressant requirements for future trials, and (4) strategies to improve postinjection graft identification.

The rapid development of a progressive postoperative kyphotic deformity in 1 of 6 patients highlights questions both as to the cause as well as to future management considerations. Published literature in the treatment of spondylotic myelopathy supports a high rate (~20%) of postsurgical kyphosis following multilevel cervical laminectomy in the adult population.[15-17] This has been observed to increase with a concomitant preoperative loss of cervical lordosis.[15] Biomechanical studies expectedly support a transition of forces from the removed posterior elements to the adjacent facet joints.[18] Although operative facet joint compromise and known loss of preoperative cervical lordosis may contribute to progressive deformity, it remains unclear as to whether ALS-associated erector spinae neuromuscular dysfunction may also be contributory. "Dropped head syndrome," progressive cervical kyphosis, is a recognized clinical sign in ALS (1.3%)[19,20] and has been attributed to erector spinae neuromuscular dysfunction. However, these data do not reflect the development of progressive deformity in ALS patients that had previously received a multilevel cervical laminectomy. It may be that performance of a multilevel laminectomy in ALS patients is more destabilizing than in patients without neuromuscular dysfunction. In this setting, a preoperative loss of cervical lordosis and the presence of underlying progressive neuromuscular dysfunction may both serve as risk factors for the development of postoperative kyphosis. We recommend obtaining preoperative static and dynamic imaging (eg, flexion-extension radiographs) to evaluate for any evidence of instability.[16] Future cervical microinjection procedures in ALS patients will include careful preservation of the facet joints and consideration of lateral mass instrumented fusion for patients with preoperative evidence of dynamic subluxation or loss of cervical lordosis. A laminectomy or placement of laminoplasty hardware may be sufficient in the absence of dynamic instability and with adequate preoperative cervical lordosis.

Perioperative complications also included 1 patient who required reintubation in the postoperative anesthesia care unit, and 1 patient who required reoperation for superficial wound dehiscence with associated infection. The patient who required perioperative intubation (group D, patient 2; trial patient 14) was the only individual who carried an ALS variant diagnosis (eg, bulbar predominant ALS). All other patients carried a diagnosis of classical ALS. In this trial, the enrollment (inclusion/exclusion)

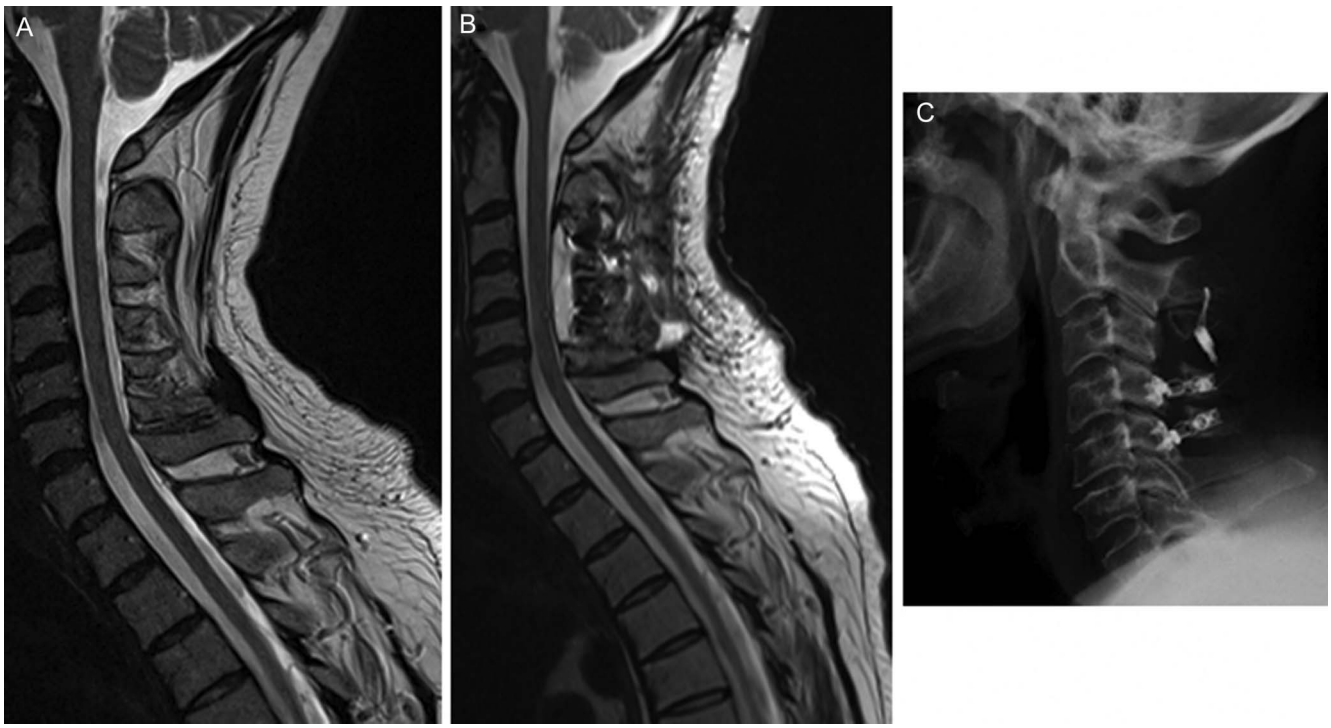

FIGURE 5. *Technique revision, laminoplasty hardware placement. Given the observation of induced postsurgical kyphotic deformity in the early cervical microinjection patients, an alteration in surgical technique was introduced for group E patients. During dissection, the posterior tension band was preserved. At the conclusion of exposure, the interspinous ligament was cut at the C2/3 level. Next, a bilateral laminectomy was performed but with preservation of the posterior tension band caudally at C5/6. To provide the visualization necessary to perform unilateral injection into the cervical spinal cord, the posterior elements of C3-C5 were placed behind the microinjection platform retractor blade. At the conclusion of microinjection and dural closure, the posterior elements were secured in place with bilateral laminoplasty plates and posterior wiring. The posterior elements of C3 were not included in this construct because the diminutive size of the bony structure precluded wiring.* **A**, *a preoperative T2-weighted midsagittal cervical MRI.* **B**, **C**, *three-month postoperative MRI and lateral plain film are shown.*

criteria did not distinguish between presenting forms of ALS. Future studies should consider the baseline dysfunction of the pharyngeal musculature in bulbar ALS to be a risk factor for compromised perioperative airway protection. This could be accommodated with plans to electively leave the patient intubated in the immediate postoperative period. Further, it may be reasonable to exclude this ALS variant from near-term prospective studies, because patients with bulbar ALS are less likely to benefit from direct delivery of a cell-based therapeutic to the spinal cord. The patient who required reoperation for wound dehiscence/infection and subsequently for hardware removal (group E, patient 3; trial patient 11) received previous bilateral thoracolumbar microinjection. As a result, this patient had been on a chronic multiagent immunosuppressant regimen. Future efforts could mitigate this risk by (1) more closely spacing the staged operations, (2) performing the thoracolumbar and cervical operations simultaneously (if both are being performed), or (3) providing an immunosuppressant holiday (reduced or withheld immunosuppressants) during the immediate perioperative healing period.

As a universally fatal neurodegenerative disease, there are no effective therapeutic interventions for ALS. Although the delivery of a cellular graft to achieve neuroprotective and neurorestorative end points is an attractive goal, little is known about the immunological environment of the human spinal cord. Therefore, the use of immunosuppression in the maintenance of a viable cellular graft is both an important consideration and holds an uncertain future role. Preclinical studies by Geron, Inc, and our group were unable to demonstrate a T-cell proliferative response to a delivered cellular graft.[10] However, on the basis of the solid organ transplantation literature, we have incorporated a conservative multiagent regimen. Tailoring or withholding of immunosuppressants (immunosuppressant holiday) will require an improved mechanistic understanding of the spinal cord postengraftment immunological microenvironment. Preclinical studies will be necessary to elucidate (1) the role of these agents in maintenance of viable cellular grafts in the spinal cord, as well as (2) the specific immunogenic mechanisms by which the grafts trigger a host response. This will complement data that exist in the solid organ transplantation literature and will ultimately result in the development of more tailored immunosuppressant regimens and cellular grafts with reduced immunogenicity. The terminal nature of ALS, and the fact that some trial patients have been unable to tolerate maintenance on the full

immunosuppressant regimen, indicates that preliminary data for the role of a multiagent immunosuppressant regimen in maintenance of a cellular graft may be provided by postmortem histological evaluation of the spinal cord graft sites. The long-term goal of these studies will be to minimize the number of agents and duration necessary for postengraftment immunosuppression when delivering a cellular graft for the treatment of ALS or alternate indications.

Six patients have died to date while enrolled in this trial. This includes 1 patient who underwent cervical microinjection. The deceased cervical microinjection patient (group D, patient 2; trial patient 14) carried a diagnosis of bulbar ALS. This patient died at 6 months postoperatively. Whereas the patient succumbed to disease progression, respiratory failure, and pneumonia, perioperative functional measures were worsened in this patient. A contribution of surgery cannot be excluded in this patient with an aggressive ALS variant. In each deceased patient, graft has been identified through a combination of precise graft site localization and the use of advanced detection techniques. Accurate graft site localization was accomplished through the use of video recordings and photographs on the intraoperative microscope. Figure 6A demonstrates a specific "vascular fingerprint" on the dorsum of the spinal cord surface as recorded by the operative microscope. Figure 6B shows a postmortem view of the same tissue, illustrating that the exact site of each cord penetration and graft can be effectively localized with the aid of this vascular fingerprint. Histological graft identification in porcine preclinical studies of multiple cell types have used species-specific markers (as human cells were delivered into swine) as well as fluorescence in situ hybridization to Y-chromosome specific markers. This was possible because graft cells were male (XY) and recipient animals were female (XX). Although 2 enrolled patients are female, the remaining 13 are male. This has provided a new and ongoing challenge toward the identification of a human cellular graft when delivered to the human spinal cord. A full histological description of graft viability and detection techniques will be separately reported.

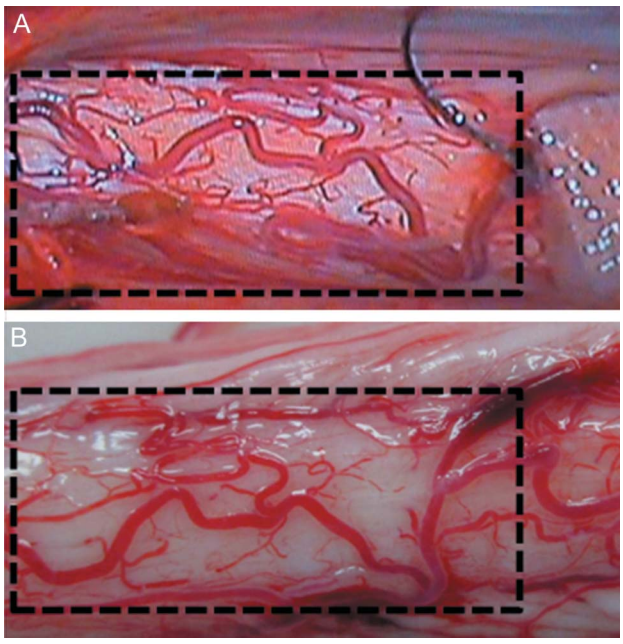

FIGURE 6. *Vascular fingerprint, graft identification. Preclinical and clinical experience supports a highly variable vascular pattern observed on the dorsal surface of the spinal cord. We have used this vascular pattern to provide landmarks during graft site identification.* **A**, *this intraoperative image, taken with the operative microscope, shows a highly specific vascular pattern and demonstrates cord penetration with the floating cannula, to the left of center in the black inset.* **B**, *the same highly specific vascular pattern is observed in the same cord specimen after removal at autopsy. This allows reorientation to the exact sites of cord penetration and graft delivery.*

CONCLUSION

Even the compromised ALS spinal cord is tolerant to serial targeted microinjections to the cervical and thoracolumbar spinal cord. Demonstration of procedural safety in this vulnerable population may prevent the need to re-create a "risk escalation" paradigm in future trials. This is especially relevant for ALS since thoracolumbar microinjection does not meet the ultimate desired endpoint of prolonging tracheostomy-free survival. The potential for post-operative spinal instability should be considered in preoperative planning. Patients with either bulbar predominant ALS or on chronic immunosuppression may be susceptible to elevated periprocedural risk. Near-term preclinical studies are needed to further develop (1) an ability to refine the role for immunosuppressants, (2) imaging modalities capable of detecting postimplantation graft localization and viability, and (3) improved techniques to detect human allogeneic grafts in a human recipient spinal cord. We have recently received FDA approval to progress into a phase II trial. This will be needed to define a dose-limiting toxicity threshold, a maximum tolerated dose for the ALS spinal cord. Further, this effort will assess the range of modifiable infusion parameters that may be tolerated (eg injection number, sites injected, total dose delivered). Once defined, subsequent multicohort dose-ranging trials designed to assess therapeutic efficacy can be undertaken. The vulnerable nature of the ALS spinal cord may serve as an ideal setting to create a conservative estimate of spinal cord tolerance when generalizing biological therapeutics delivery approaches to alternate indications. Long-term efforts will focus on the validation of a targeted direct microinjection approach for application to the spectrum of spinal cord afflictions.

Disclosures

Neuralstem, Inc, provided financial assistance for microinjection platform construction and is funding the phase I clinical trial that is described herein. Dr Boulis has received an inventors' fee for the microinjection platform and floating cannula. He is also eligible for royalties associated with future licensing of these technologies. Dr Glass has received research funding from Neuralstem, Inc. Dr Feldman has received support from Novartis Pharmaceuticals for services as a consultant. Dr Johe is an employee at Neuralstem, Inc. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

1. Xu L, Yan J, Chen D, et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation*. 2006;82(7):865-875.
2. Guo X, Johe K, Molnar P, Davis H, Hickman J. Characterization of a human fetal spinal cord stem cell line, NSI-566RSC, and its induction to functional motoneurons. *J Tissue Eng Regen Med.* 2010;4(3):181-193.
3. Riley J, Taub J, Raore B, Boulis NM. An overview of domestic and international clinical trials for delivery of cellular therapies to the spinal cord. *Clin Neurosurg.* 2012;59:98-104.
4. Raore B, Federici T, Taub J, et al. Cervical multilevel intraspinal stem cell therapy: assessment of surgical risks in gottingen minipigs. *Spine (Phila Pa 1976).* 2011;36 (3):E164-E171.
5. Riley J, Hurtig CV, Boulis N. Translating biologic therapies from bench to bedside for amyotrophic lateral sclerosis. *Personalized Med.* 2012;9(6):645-655.
6. Riley JP, Raore B, Taub JS, Federici T, Boulis NM. Platform and cannula design improvements for spinal cord therapeutics delivery. *Neurosurgery*. 2011;69(suppl 1): ons147-ons155.
7. Riley J, Federici T, Park J, et al. Cervical spinal cord therapeutics delivery: preclinical safety validation of a stabilized microinjection platform. *Neurosurgery*. 2009;65(4):754-761; discussion 761-752.
8. Federici T, Riley J, Park J, Bain M, Boulis N. Preclinical safety validation of a stabilized viral vector direct injection approach to the cervical spinal cord. *Clin Transl Sci.* 2009;2(2):165-167.
9. Nematalla AH, Bakr MA, Gheith OA, Elagroudy AE, Elshahawy el M, Aghoneim M. Steroid-avoidance immunosuppression regimen in live-donor renal allotransplant recipients: a prospective, randomized, controlled study. *Exp Clin Transplant.* 2007;5(2):673-679.
10. Riley J, Federici T, Polak M, et al. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I safety trial, technical note, and lumbar safety outcomes. *Neurosurgery*. 2012;71(2):405-416.
11. Glass JD, Boulis NM, Johe K, et al. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: results of a phase I trial in 12 patients. *Stem Cells*. 2012;30(6):1144-1151.
12. Boulis NM, Federici T, Glass JD, Lunn JS, Sakowski SA, Feldman EL. Translational stem cell therapy for amyotrophic lateral sclerosis. *Nat Rev Neurol.* 2011;8(3):172-176.
13. Xu L, Ryugo DK, Pongstaporn T, Johe K, Koliatsos VE. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. *J Comp Neurol.* 2009; 514(4):297-309.
14. Federici T, Hurtig CV, Burks KL, et al. Surgical technique for spinal cord delivery of therapies: demonstration of procedure in gottingen minipigs. *J Vis Exp.* 2012; (70):e4371.
15. Kaptain GJ, Simmons NE, Replogle RE, Pobereskin L. Incidence and outcome of kyphotic deformity following laminectomy for cervical spondylotic myelopathy. *J Neurosurg.* 2000;93(2 suppl):199-204.
16. Guigui P, Benoist M, Deburge A. Spinal deformity and instability after multilevel cervical laminectomy for spondylotic myelopathy. *Spine (Phila Pa 1976).* 1998;23 (4):440-447.
17. Albert TJ, Vacarro A. Postlaminectomy kyhposis. *Spine (Phila Pa 1976).* 1998;23 (24):2738-2745.
18. Pal GP, Sherk HH. The vertical stability of the cervical spine. *Spine (Phila Pa 1976).* 1988;13(5):447-449.
19. Gourie-Devi M, Nalini A, Sandhya S. Early or late appearance of "dropped head syndrome" in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry.* 2003;74 (5):683-686.
20. Ringel SP. Clinical presentations in neuromuscular disease. In: Vinken PJ, Bruyn GW, eds. *Handbook of Clinical Neurology*. New York: Elsevier; 1979: 295-348.

Supplemental digital content *is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.neurosurgery-online.com).*

Acknowledgments

We would like to thank all study participants who have graciously shared in our collective desire to overcome amyotrophic lateral sclerosis. Neuralstem, Inc, has provided study funding and has purchased an exclusive license to the spinal cord microinjection apparatus, of which Dr Boulis is the inventor.