Title: Intraspinal Stem Cell Transplantation for Amyotrophic Lateral Sclerosis

Running head: Stem Cell Transplantation for ALS

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder in which the loss of upper and lower motor neurons produces progressive weakness and eventually death. In the decades since the approval of riluzole, the only FDA approved medication to moderately slow progression of ALS, no new therapeutics have arisen to alter the course of the disease. This is partly due to our incomplete understanding of the complex pathogenesis of motor neuron degeneration. Stem cells have emerged as an attractive option in treating ALS since they come armed with equally complex cellular machinery and may modulate the local microenvironment in many ways to rescue diseased motor neurons. While various stem cell types are being evaluated in preclinical and early clinical applications, here we review the preclinical strategies and advances supporting the recent clinical translation of neural progenitor cell therapy for ALS. Specifically, we focus on the use of spinal cord neural progenitor cells and the pipeline starting from preclinical studies to the designs of the Phase I and IIa clinical trials involving direct intraspinal transplantation in humans.

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective and progressive degeneration of upper and lower motor neurons, with an estimated cost to society ranging from $256 million to $433 million in the United States alone.[1] ALS manifests as an insidious, inexorable decline in motor function, with progressively compromised strength, coordination, gait, and respiratory function, leading to death within an average of 3-5 years from diagnosis.[2] Approximately 15% of cases are associated with germline mutations in a number of genes, including Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1), transactive response DNA-binding protein 43 (TDP43), fused in sarcoma (FUS), and the more recently described hexanucleotide repeat expansions in the chromosome 9 open reading frame 72 (c9orf72).[3] The vast majority of ALS cases, however, are sporadic, and the underlying pathophysiology remains unclear.

Many hypotheses exist to explain motor neuron death in ALS, including excitotoxicity,[4-8] loss of neurotrophic factors,[9-11] inflammatory signaling,[12] mitochondrial pathology,[13, 14] and endoplasmic reticulum dysfunction and protein misfolding,[15] among others.[16] This complexity has stood in the way of successful development of mechanism-based pharmaceutical treatments, and riluzole, a drug that extends survival by mere months, remains the only United States Food & Drug Administration (FDA)-approved therapy for ALS.[17] Disappointingly, a number of trials targeting some of the above-mentioned pathways have failed in large-scale clinical trials.[18-36]

Given the multifaceted nature of ALS, stem cell-based therapy has recently become an attractive option. Initially proposed as a means for motor neuron replacement, stem cells may actually provide a number of benefits by modulating the local microenvironment to facilitate native motor neuron survival. Stem cells elaborate neurotrophic factors such as glial-derived neurotrophic factors (GDNF), brain-derived neurotrophic factor (BDNF), vascular endothelial

growth factor (VEGF), and insulin-like growth factor-I (IGF-I).[37, 38] Certain stem cells can also differentiate into astrocytes and increase efficiency of glutamate re-uptake, a process that is disrupted in ALS.[39] Furthermore, stem cells that form neuronal cells may form synapses onto native motor neurons and provide trophic and/or contact-mediated support.[40]

In translating a stem cell-based approach from bench top studies to clinical trials, a number of criteria must be achieved. First, the appropriate type of stem cell must be identified and be obtainable in numbers that can be used therapeutically. Second, the means of cell delivery must be carefully considered, balancing the risks of invasive procedures with the need to deliver sufficient cells to specific areas within the nervous system. Here, we review the strategy of modulating the motor neuron microenvironment using cellular-based techniques and briefly introduce the current options being developed as cellular therapies. We then emphasize the preclinical data supporting our own journey toward a clinical trial using neural progenitor cells (NPCs) in ALS, and finally describe the results of our Phase I trial, outline the Phase IIa trial which has recently concluded, and offer perspective on the future of stem cell-based treatment for ALS.

STEM CELLS: MODULATING THE LOCAL MICROENVIRONMENT

The possibility of cellular replacement generated considerable enthusiasm for stem cell applications in neurodegenerative diseases, including ALS. Early data fueled this fervor, with studies in chicks showing the ability of stem cells to differentiate into motor neurons and reinnervate muscle.[41] This was then applied to rodent models of ALS bearing the first described familial ALS mutation (SOD1^{G93A}) and exhibiting a phenotype of progressive motor neuron loss and weakness[42, 43]; however, it quickly became apparent that hurdles for motor neuron

replacement included not only introduction of stem cells into the spinal cord without damage to surrounding neural tissue, but also efficient differentiation into motor neurons, integration with local circuitry, growth of new axons in the mature central nervous system (CNS), proper axonal guidance to correct musculature, formation of mature neuromuscular junctions, and sufficient pruning for functional activity.[44] At this time, these hurdles cannot be overcome with our current technologies.

For true reconstruction of the motor system, biotechnology must advance to a point where implanted stem cells can receive new synaptic contacts as well as sprout new axons, typically impermissible in the CNS. These new axons must then be coaxed to enter ventral roots and follow a "bread crumb trail" of neurotrophic factors to create *de novo* neuromuscular junctions. While early work to achieve this has been attempted,[45, 46] the difficulties in translating these strategies to clinical practice have been prohibitive, especially considering the short survival window for ALS patients. Some strategies to address or bypass some of these barriers capitalize on intact transport functions of the nervous system; for example, targeting skeletal muscle allows neurotrophic factors, nucleic acids, or viral vectors to be brought to the appropriate motor neurons in the ventral horn *via* retrograde transport.[47-51] Similarly, intracranial injections of stem cells have been attempted to widely affect the motor system in an anterograde fashion.[52-54] These multimodality and multisite strategies, combined with novel biomaterials that carry various biologics and new techniques for delivery (e.g. ever miniaturized robotics or advanced image-guided intervention) could make motor neuron replacement a real strategy in the future.

For now, despite the selective vulnerability of alpha motor neurons in ALS, evidence has accumulated to suggest that ALS is not solely a disease of motor neurons, but rather one in

which the local "neighborhood" contributes to motor neuron demise.[55-57] Elegant experiments performed in chimeric mutant SOD1 mice showed that motor neuron death was linked to surrounding non-neuronal genotype rather than intrinsic properties of the neurons themselves: normal motor neurons surrounded by mutant SOD1-expressing non-neuronal cells displayed characteristics of degeneration akin to that seen in ALS, whereas mutant SOD1-expressing motor neurons surrounded by wild-type non-neuronal cells were protected from cell death.[58] Again, the exact mechanisms of this are unclear, but it is now fairly well-established that motor neuron interaction with surrounding neurons, astrocytes, vasculature, skeletal muscle, microglia, and other immune cells contribute to motor neuron death.[6, 59-62] Stem cells enter this scene armed with the full armamentarium of cellular processes (neurotransmitter uptake, synapse formation, inflammatory signaling, neurotrophic factor signaling, etc.) that can ameliorate toxic environments in a multifactorial fashion, a process difficult to achieve by small molecule therapy alone. Indeed, as pharmacologics advance, small molecule therapies may work synergistically with stem cells, and the combination could form the basis for future paradigms in clinical trial design.

There are many options to consider when developing a stem cell-based cellular therapy. This includes selection of the optimal stem cell type, and recent studies have focused on the potential utility of embryonic stem cells, olfactory ensheathing cells,[63-66] peripheral blood stem cells,[53, 67-73] adipose stem cells,[74] bone marrow-derived mesenchymal stem cells,[75-81] and NPCs.[82] Determination of the optimal therapeutic delivery paradigm is also pertinent, and approaches range from systemic mobilization or intravenous delivery to precise localized delivery strategies within the CNS. Recent progress detailing the preclinical development and early clinical translation of these varying therapeutic strategies are reviewed elsewhere,[83-86] and these advances

collectively provide important insights into the potential safety, feasibility, and preliminary efficacy of the stem cell therapies currently being considered to treat ALS around the world; however, here we present our recent journey developing and translating a spinal cord NPC-based therapy for ALS.

SPINAL CORD NPCs

The Path Towards Clinical Testing

NPCs are pluripotent cells that have undergone the initial stages of differentiation, such that the cell types that arise are limited to neuronal and glial lineages. Over the past decade, our recent preclinical validation and clinical translation efforts have focused on the utilization of NSI-566RSC, an established NPC cell line generated from donated fetal spinal cord tissue.[38, 40, 87] While mesenchymal stem cells obtained from bone marrow, peripheral blood, umbilical cord blood and adipose tissue can be expanded and transplanted autologously with less risk of rejection, these cells cannot recapitulate neuronal synapses that might be crucial for rescuing motor neurons. Also, NPCs obtained from embryonic tissue have been spared the possible disease-related environmental exposure and epigenetic changes seen by the patient. However, on the other side of the spectrum, part of the risk of using more primitive embryonic stem cells or even olfactory ensheathing cells is ongoing proliferation and formation of teratomatous tumors.[88, 89] Thus, while a disadvantage of using a NPC line is the immunosuppression required, the benefit comes from the ability of "fresh" NPCs to generate CNS-relevant cell types, form synapses, and still possess a decreased "tumorigenic" profile.

Initial preclinical studies assessing the therapeutic potential of the NSI-566RSC line in SOD1^{G93A} rats showed that transplanted stem cells rescued motor neurons, improved motor

function, and prolonged lifespan.[38, 40, 90, 91] These studies further supported a mechanism whereby the transplanted cells modulated the local microenvironment by forming synapses with host motor neurons and eliciting neurotrophic signaling that rescued motor neurons only in the area of stem cell injection.[38, 40] Notably, studies targeting both lumbar and cervical spinal cord segments in SOD1^{G93A} mice demonstrated that dual targeting conferred greater therapeutic benefit.[87]

Further preclinical assessments along the pathway toward clinical translation included verification of the safety of intraspinal stem cell injection in a large animal. Intraspinal injection carries the greatest degree of risk for neurologic damage, thus limiting interventions to defined segments of the CNS. However, in contrast to intravascular or intrathecal administration, direct injection ensures localization of viable cells in the region of interest (here, the ventral horn of spinal cord). Thus, the potential for local growth factor production and synapse formation can be maximized, while at the same time bypassing the blood brain barrier. Studies performed by a team led by Dr. Nicholas Boulis at Emory University proved critical in validating this approach, as delivery of a payload of cells by direct injection was a daunting task considering that accurate needle placement must be made into the anterior horn without damaging the exquisitely sensitive surrounding structures of the spinal cord. Added to this is the fact that this microscopic target moves and pulsates in response to the patient's variable heart rate and respirations. Thus, a specialized spinal cord injection device was devised (Figure 1).[84] The device is anchored to the patient's own bony anatomy and therefore grossly moves with the patient, enhancing safety if an adjustment of the operative table is needed or if the patient exhibits any intra-operative movement. A "gondola" affixed to this anchored frame carries a multi-axial "Z-drive", allowing the surgeon to precisely angulate and space each injection. The injection needle design is based on pre-operative imaging with a stop such that when the hub is at the dorsal spinal cord surface,

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the needle length (3-5 mm, based on distance to anterior horn as measured on pre-operative MRI) places the injection tip in the anterior horn. This injection needle is connected to flexible tubing, allowing the needle to "float" and move with the pulsations of the spinal cord without causing unnecessary trauma.

Iterations of this device were designed for targeting the cervical or lumbar spinal cord segments and were then tested and optimized in the Gottingen minipig.[92-94] These experiments showed that minipigs tolerated 5 or 10 unilateral injections (6 μL per injection) in cervical spinal cord. Most animals recovered motor and sensory function within 6 days of surgery and all recovered to preoperative baselines by postoperative day 14. Initial immunosuppression protocols using tacrolimus and methylprednisolone were also optimized in these experiments to maximize xenogeneic cell survival. These studies not only demonstrated that injected cells were accurately placed within the anterior horn of the spinal cord using the spinal delivery frame (Figure 1),[84] but most importantly, that the animals recovered with robust limb motor function after their surgical procedures, providing confidence that such an approach could be successful in man.

A Phase I Clinical Trial

Preclinical evidence of NPC transplantation efficacy in the SOD1[G93A] rat along with demonstration of the feasibility and safety of spinal cord injection using the spinal injection device in the minipig supported approval from the FDA for the trial "A Phase l, Open-label, First in Human, Feasibility and Safety Study of Human Spinal Cord Derived Neural Stem Cell Transplantation for the Treatment of Amyotrophic Lateral Sclerosis" (NCT01348451). Given the novelty of this intervention, the trial utilized a "risk escalation" design in terms of subject disease severity, intervention target, and injection numbers (Table 1). Dr. Jonathan Glass at Emory University

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coordinated a team of Neurologists and Neurosurgeons to test this novel approach.[82] Group A included non-ambulatory patients who received 5 unilateral (n = 3 subjects) or 10 bilateral (n = 3 subjects) injections in the lumbar spinal cord (L2-4), whereas Group B (n = 3 subjects) and C (n = 3 subjects) included ambulatory subjects who received 5 unilateral or 10 bilateral injections into the lumbar spinal cord, respectively. Group D (n = 3 subjects) included ambulatory patients who received 5 unilateral cervical spinal cord injections (C3-5). Notably, these cervical injections target the motor neurons innervating the diaphragm, thus offering a means to offer protection against the most common cause of death in ALS – respiratory failure. The 3 patients in group C then received 5 unilateral cervical spinal cord injections in addition to their lumbar injections and formed Group E. To ensure survival of the transplanted stem cells, patients received methylprednisolone and basiliximab at the time of surgery, and were maintained post-operatively using another dose of basiliximab, a prednisone taper, and maintenance tacrolimus and mycophenolate mofetil. Further details of the clinical trial as well as the surgical implantation technique have been described elsewhere.[84, 95-98]

To date, 7 deaths have been recorded among the patients enrolled in the Phase I trial; 6 due to progression of the disease and one due to a previously undiagnosed congenital cardiac defect. Autopsy studies on postmortem spinal cord tissue from these subjects have provided exciting data to support the feasibility of surgical implantation of NPCs in ALS. First, in terms of safety, no tumor formation was evident in any subject. Second, histological assessments revealed nests of live cells representative of the transplanted NPCs in the regions targeted by the transplants, and male donor cells were evident in the female transplant recipients (Figure 2).[99] Moreover, every patient at autopsy demonstrated the persistence of donor-specific DNA in injected spinal cord when assayed for donor HLA genotype by qPCR (Figure 3).[99]

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In the Phase I trial, while a number of adverse events (AEs) were observed, most were related to the immunosuppression regimen initiated to ensure engraftment of implanted stem cells[82] and no AEs were related to the surgical procedure,[97, 98] emphasizing the safety and tolerability of this cellular therapy and transplantation technique. Furthermore, clinical assessments including ALS functional rating scale-revised (ALSFRS-R), ALS quality of life (ALSQOL), forced vital capacity (FVC), hand-held dynamometry (HHD), and grip strength testing (GST) showed no acceleration of disease course. In fact, although the phase I study was not designed to establish efficacy, the slope of disease progression appeared to improve after surgical implantation when compared to progression rate prior to surgery, particularly in group C/E patients (Figure 4).[83] Furthermore, in the majority of patients, GST, HHD, and electrical impedance myography outcomes at 9, 12 and 15 months were improved when compared to pre-surgical baselines.[83] These results generated a great deal of excitement and allowed the transition to a Phase IIa trial.

A Phase IIa Clinical Trial

Given these encouraging results of the Phase I safety trial, the FDA approved the Phase IIa trial, "A Phase II, Open-label, Dose Escalation and Safety Study of Human Spinal Cord Derived Neural Stem Cell Transplantation for the Treatment of Amyotrophic Lateral Sclerosis" (NCT01730716). This trial was designed to assess the maximum tolerated dose of cells, measuring the same clinical assessments as the Phase I trial, and treatment groups progressively received increasing doses of cells via intraspinal cord injections (Table 2). Subjects in Group A (n = 3) received 5 bilateral (10 total) cervical injections for a total of 2 million cells. Subjects in Groups B, C, and D (n = 3 each) then received 10 bilateral (20 total) cervical injections of

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increasing cell doses for a total of 4, 6, and 8 million cells, respectively. Finally, subjects in Group E (n = 3) received 8 million cells in 10 bilateral (20 total) cervical injections, followed a month later by a subsequent dose of 8 million cells in 10 bilateral (20 total) lumbar spinal cord injections, resulting in a total of 16 million cells. A training program for participating Neurosurgeons on operation of the spinal injection system was implemented prior to extending the trial to new sites, and this standardization will be continued in future trials.

The final surgery was performed in July 2014 and data review is ongoing; however, the preliminary results are promising and ~~we are currently planning~~ the next Phase II/III trial is in the early planning phase. Aspects of trial design that are currently under discussion include inclusion/exclusion criteria, the possibility of inclusion of experimental disease biomarkers, need for immunosuppression, appropriate primary outcome measures, as well as the feasibility and ethics of a placebo control group. Based on experience in the Phase I and Phase IIa trials, as well as the theory that stem cells improve the motor neuron microenvironment, it is anticipated that patients with early-stage disease would likely benefit the most. Therefore, early-stage patients without bulbar involvement will likely be the major target population in any future efficacy study. Important outcome measures include pulmonary function tests as well as assessment of motor strength, in addition to survival data.

However, these outcome measures may be meaningless without a group for comparison. Historical controls from previous ALS trials would likely be insufficiently matched to treatment groups in this stem cell paradigm. In an ideal world, any measure of therapeutic efficacy should be compared with a placebo control group. Particularly in surgical trials, the placebo effect can be strong, as underscored by recent cellular therapy approaches for Parkinson's Disease.[100] Here lies the crux of an ethical dilemma that accompanies stem cell trials: in a rapidly fatal disease

such as ALS, is it ethical to subject patients to the risks of a sham surgical procedure and immunosuppression for the sake of investigative rigor? Varying lead-in phases could be utilized, but, given the progressive nature of the disease, patients receiving stem cell transplants at a later stage will likely be a very different population than those receiving stem cells upfront. Placebo surgery could be an option, with increasing degrees of risk having been proposed: from a mere general anesthetic, to skin incision only, to removal only of spinous processes, even up to full injections with a vehicle control. The tension between scientific harmony and clinical nonmalfeasance will have to be carefully negotiated as these trials move forward.

CONCLUSIONS: THE HORIZON

The journey from the initial studies utilizing stem cells in preclinical models of ALS to the completion of the Phase I and Phase IIa trials has been long and storied, yet immensely rewarding. While we have focused on one series of trials utilizing NPCs, the potential benefits of cell-based therapy in ALS are being actively studied internationally. Many trials focus on autologous, intrathecal administration of bone marrow-, umbilical cord-, or adipose-derived mesenchymal stem cells, such as trials in China (NCT01494480), Poland (NCT02193893), Iran (NCT02116634, NCT02492516), South Korea,[101] India (NCT02242071), and at the Mayo Clinic, USA (NCT01609283). A paradigm from Brainstorm-Cell Therapeutics modifies autologous mesenchymal stem cells to secrete a variety of neurotrophic factors prior to intramuscular and intrathecal co-implantation (NCT02017912).[78] Similarly, Spanish groups have performed intraspinal injection of autologous mesenchymal stem cells,[76] and are comparing intravenous, intramuscular, intraspinal, and intrathecal therapy (NCT01254539, NCT02286011, NCT02290886). A planned trial by Q Therapeutics Inc. utilizes the technique of intraspinal

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injection described here for the introduction of glial-restricted neural progenitor cells (NCT02478450).

~~Continued~~ Moving forward, continued evaluation of these trials as well as other maturing trials for ALS (reviewed elsewhere)[83, 85, 86, 102] should consider some basic tenets of stem cell therapy: 1) stem cells should be readily obtainable in numbers sufficient for clinical use, 2) cells should minimize the potential for tumor formation yet survive in sufficient numbers to benefit motor neurons, 3) the paradigm for cell delivery should balance CNS penetration and therapeutic potential with procedural reproducibility and safety, 4) clinical trial design should be robust with sufficient subjects to glean meaningful data, yet be sensitive to the varied presentation and course of disease, 5) trial outcomes should remain objective with measurements such as the ALSFRS-R and pulmonary function, and 6) subsequent analysis should include delineation of the survival, function, and potential mechanism of transplanted cells. Also relevant to future trial planning is the projected cost of this therapy. While accurate estimates cannot be provided given the early investigative aspect of these trials, the bulk of costs will likely be associated with the stem cell product, surgical and perioperative costs, as well as any ongoing immunosuppression. For the Phase I and IIa NPC transplantation trials, the stem cell product was provided at no cost by Neuralstem, Inc. Given the heterogeneity of the modern healthcare landscape, the hospital and procedural fees associated with intraspinal injection, post-operative hospitalization, and ongoing immunosuppression will vary from center to center. Certainly, the cost of the stem cells as well as the added surgical and medical costs associated with this procedure will need to be balanced with the estimated annual cost of disease, which is about $63,693 per year, per patient.[1]

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These collective tenets and considerations will be heavily weighed as we enter into planning the next phase of our clinical trial and must certainly be considered with the continued translation and evaluation of other cellular therapy paradigms as well.

In addition to the data obtained thus far in these and other stem cell clinical trials, the nascent ideas being nurtured in the laboratory also continue to inform the future of cell-based therapy for ALS. Particularly, much excitement surrounds applications harnessing the potential of induced pluripotent stem (iPS) cells, as the ability to de-differentiate adult fibroblasts into pluripotent stem cells confers a range of exciting and newly attainable possibilities. Most immediately, motor neurons derived by iPS technology from donated ALS patient tissue will allow *in vitro* study of disease mechanisms and high-throughput screening of potential therapeutics[103-105]. Furthermore, healthy individuals, and even ALS patients themselves, now become potential donors of stem cells that could be used in the study and treatment of ALS.[106, 107] At this time, although iPS cells would negate the need for immunosuppression, the logistics and cost of dedifferentiating fibroblasts, expanding a stem cell population, and differentiation prior to reimplantation (to prevent teratoma formation) within a time window prior to ALS progression is still prohibitive and at times inconsistent. However, as this and other technologies mature, iPS-based therapy may become a reality in the near future. Thus, in the stem cell era, simultaneous progress in both clinical and laboratory settings are advancing the field by providing patients with a therapy that may truly modify the course of disease while also gaining insight into the mechanisms by which stem cells provide therapeutic benefit, respectively. With this cycle between the laboratory and the clinic ongoing, stem cell therapy holds promise not only in ALS, but indeed can be implemented in a breadth of other neurologic conditions for which we can hold an immense degree of optimism.

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AUTHOR CONTIBUTIONS

KSC and ELF contributed to concept and study design; KSC, SAS and ELF contributed to drafting the manuscript and figures.

CONFLICTS OF INTEREST

The authors declare no conflict of interest with relevance to the current study.

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FIGURE LEGENDS

Figure 1: Spinal cord injection system for intraspinal stem cell transplantation. (A) Platform consists of two bridge rails (blue) and is anchored to the spine. Gondola (green) travels in a cranial-caudal dimension and compensates for slight movements in the platform application. Mechanical Z drive (orange) allows precise raising and lowering of a floating cannula. (B) Cannula tip is positioned 1 mm medial to dorsal root entry zone. (C) Needle penetrates into spinal cord ~4 mm from pial surface. (D) Once needle tip is at the target, metal outer sleeve is pulled up, allowing flexible tubing to accommodate cardiorespiratory pulsations of spinal cord. Reproduced with permission from Boulis et al., Nat Rev Neurosci, 2011.[84]

Figure 2. Donor NPC localization and characterization using XY chromosome FISH and IHC, respectively, in a female ALS patient. H&E staining shows nests of cells in the female spinal cord (A) (circle). High-power image corresponding to the nest of cells outlined in (A) is shown in (B). Sections stained with GFAP show lack of labeling of in nest of cells (C). FISH labeling shows numerous X (red) Y (green)–positive donor cells counterstained with DAPI (blue) (D). Asterisks show XX–positive recipient cells in the surrounding regions. Inset image from (D) is shown in (E). Donor NPCs are positive for XY (solid arrow). H&E labeling of NPCs graft (arrow) (F) label with SOX2 and (G) and NeuN (H). Scale bars: 1 mm (A), 50 μm (B–D), 10 μm (E), 100 μm (F–H). NPC, neural progenitor cell; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ALS, amyotrophic lateral sclerosis; GFAP, glial fibrillary acidic protein. Reproduced with permission from Tadesse et al, Ann Clin Transl Neurol, 2014.[99]

Figure 3: Identification of donor DNA in ALS spinal cord. Schematic showing presence of donor genomic DNA from spinal cord autopsy samples in six patients (1–6) as determined by

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quantitative PCR. The numbers adjacent to each schematic cord represent the percentage of

donor DNA in that tissue homogenate. Human NPCs were unilaterally injected in the lumbar

spinal cord in Patients 1, 4, and 5, bilateral lumbar in Patients 2 and 3, and unilateral cervical in

Patient 6. The black bar identifies the region containing the highest percentage of donor DNA,

ranging from 0.67% to 5.4%. ALS, amyotrophic lateral sclerosis; HSSCs, human spinal cord–

derived stem cells; NPC, neural progenitor cell; PCR, polymerase chain reaction. Reproduced

with permission from Tadesse et al, Ann Clin Transl Neurol, 2014.[99]

Figure 4: Analysis of potential windows of NPC biological activity in Subjects 10 to 12.

Postsurgery data points for Group E subjects were divided into a series of 9-month windows

beginning each month post implantation, and slopes were calculated across each window using

ALSFRS-R and compared to presurgical windows. (A) The top panel demonstrates ALSFRS-R

scores for Group E subjects during the presurgical period (green) and representative ranges

associated with the various sliding postsurgical 9-month windows (dark blue). The bottom panel

demonstrates the slopes obtained for each sliding window, with the x-axis corresponding to the

first month for each 9-month window. The first plotted slope for each subject corresponds to

their presurgical progression rate. Slope values higher than the presurgical slope at baseline

represent improved or attenuated progression rates during the designated window. Note that the

starting month of the final sliding window for each patient coincides with the dates of the second

surgery, which occur at 17.5, 19, and 16.6 months after the initial Cohort C surgery (time 0) for

Subjects 10, 11, and 12, respectively. (B) The presurgical slope and postsurgical slopes

associated with the window correlating to the peak benefit windows for both the lumbar and

cervical postsurgery time frames are summarized. ALSFRS-R, Amyotrophic Lateral Sclerosis

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Functional Rating Scale-Revised Reproduced with permission from Feldman et al, Ann Neurol, 2014.[83]

TABLES

Table 1: Risk-escalation paradigm for Phase I trial of NPCs in ALS.				
Group	**Number enrolled**	**Number of injections**	**Target**	**Final cell dose**
A1	3	5 total (5 unilateral)	Lumbar cord	5×10^5
A2	3	10 total (5 per side)	Lumbar cord	1×10^6
B	3	5 total (5 unilateral)	Lumbar cord	5×10^5
D	3	5 total (5 unilateral)	Cervical cord	5×10^5
C/E	3	15 total (5 per side lumbar) (5 unilateral cervical after observation period)	Lumbar cord Cervical cord after observation period	1×10^6 5×10^5 after observation period

Table 2: Dose-escalation paradigm for Phase IIa trial of NPCs in ALS.				
Group	**Number enrolled**	**Number of injections**	**Target**	**Final cell dose**
A	3	10 total (5 per side)	Cervical cord	2×10^6
B	3	20 total (10 per side)	Cervical cord	4×10^6

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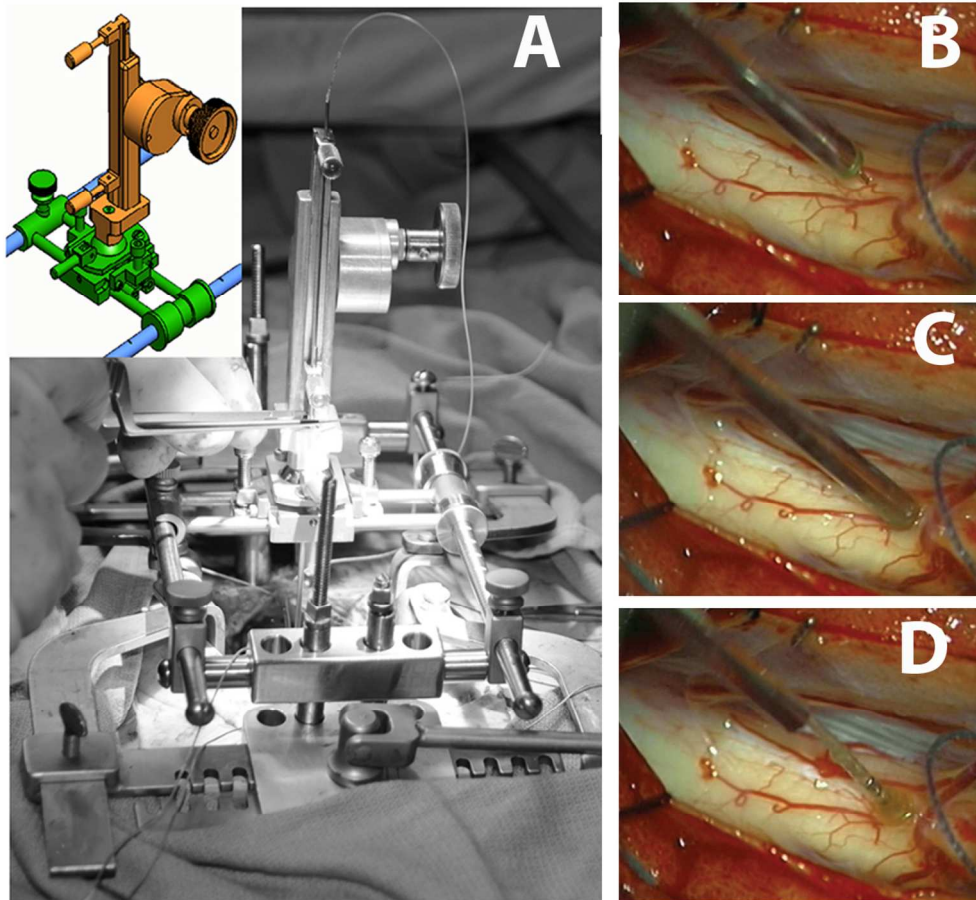| C	3	20 total (10 per side)	Cervical cord	6×10^6
D	3	20 total (10 per side)	Cervical cord	8×10^6
E	3	20 total (10 per side) for each target, staged.	Cervical cord, followed by lumbar cord	16×10^6

Figure 1. Spinal cord injection system for intraspinal stem cell transplantation. (A) Platform consists of two bridge rails (blue) and is anchored to the spine. Gondola (green) travels in a cranial-caudal dimension and compensates for slight movements in the platform application. Mechanical Z drive (orange) allows precise raising and lowering of a floating cannula. (B) Cannula tip is positioned 1 mm medial to dorsal root entry zone. (C) Needle penetrates into spinal cord ~4 mm from pial surface. (D) Once needle tip is at the target, metal outer sleeve is pulled up, allowing flexible tubing to accommodate cardiorespiratory pulsations of spinal cord. Reproduced with permission from Boulis et al., Nat Rev Neurosci, 2011.
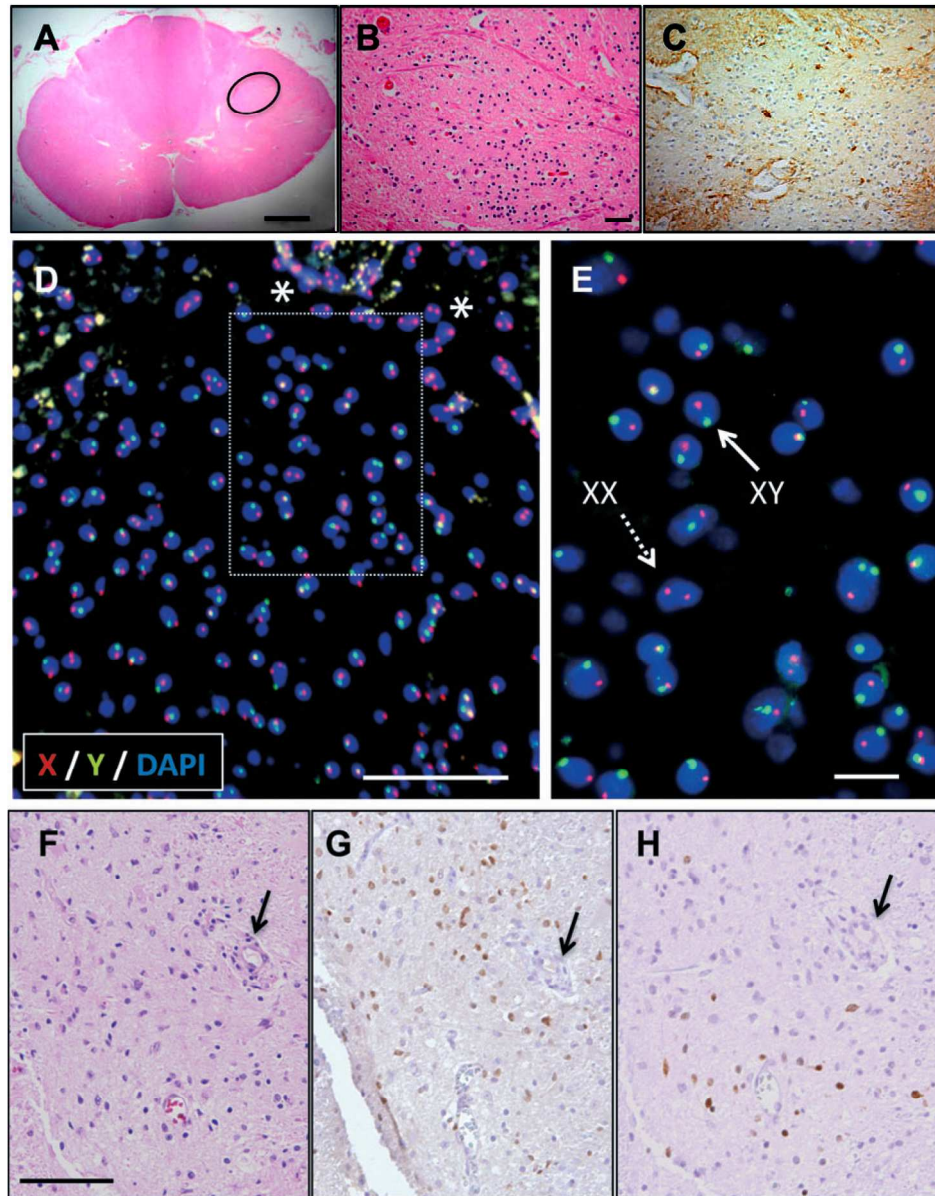111x103mm (300 x 300 DPI)

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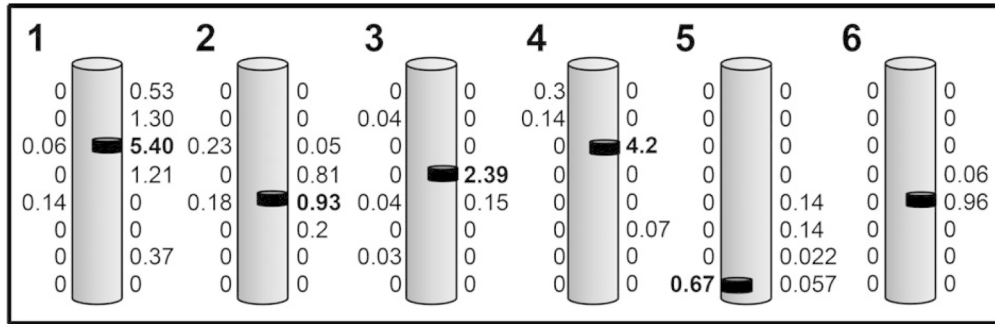728x932mm (72 x 72 DPI)

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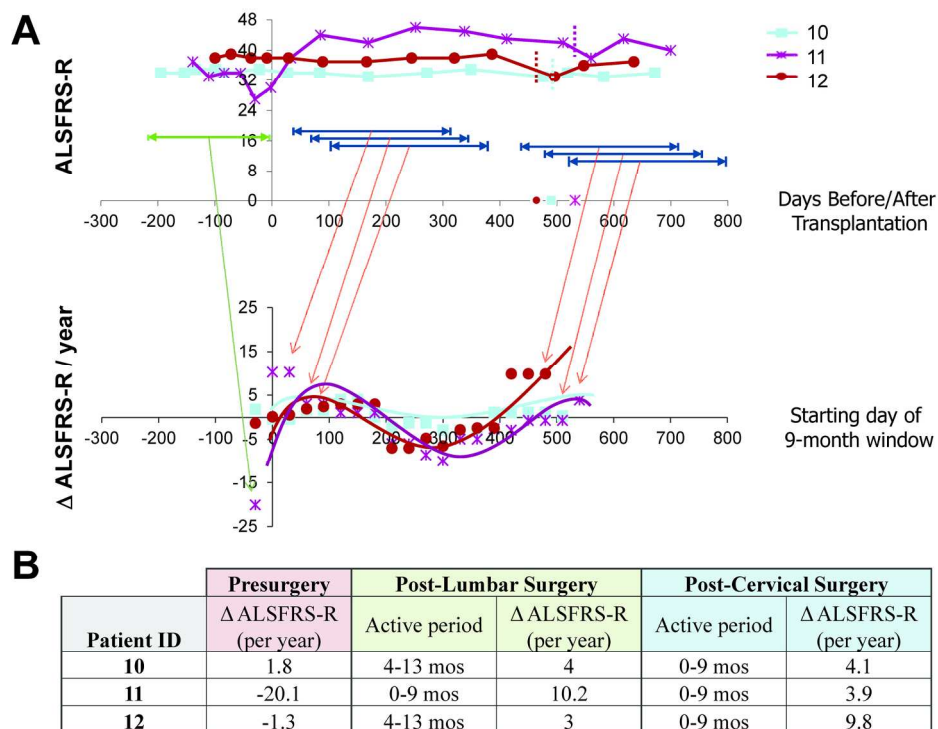731x238mm (72 x 72 DPI)

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187x143mm (300 x 300 DPI)