Transplantation of Neural Progenitor Cells Expressing Glial Cell Line-Derived Neurotrophic Factor into the Motor Cortex as a Strategy to Treat Amyotrophic Lateral Sclerosis

GRETCHEN M. THOMSEN, a,b PABLO AVALOS, a ANNIE A. MA, a MOR ALKASLASI, a NOELL CHO, a LIVIA WYSS, b JEAN-PHILIPPE VIT, b,c MARLESA GODDY, a PATRICK SUEZAKI, a OKSANA SHELEST, a KRYS TOF S. BANKIEWICZ, d CLIVE N. SVENDSEN a,b

Key Words. Amyotrophic lateral sclerosis • Stem cell transplantation • Neural progenitor cells • Gene therapy • Glial cell line-derived neurotrophic factor • Cell therapy

ABSTRACT
Early dysfunction of cortical motor neurons may underlie the initiation of amyotrophic lateral sclerosis (ALS). As such, the cortex represents a critical area of ALS research and a promising therapeutic target. In the current study, human cortical-derived neural progenitor cells engineered to secrete glial cell line-derived neurotrophic factor (GDNF) were transplanted into the SOD1G93A ALS rat cortex, where they migrated, matured into astrocytes, and released GDNF. This protected motor neurons, delayed disease pathology and extended survival of the animals. These same cells injected into the cortex of cynomolgus macaques survived and showed robust GDNF expression without adverse effects. Together this data suggests that introducing cortical astrocytes releasing GDNF represents a novel promising approach to treating ALS.

INTRODUCTION
Amyotrophic lateral sclerosis (ALS) is defined by the loss of both upper and lower motor neurons leading to rapid paralysis and death [1]. In animal models of a genetic form of the disease with mutations in the SOD1 gene [2], corticospinal motor neurons undergo early changes in structure and excitability that might underlie downstream degeneration [3–5]. Hyperexcitability of the cortex has also been reported in ALS patients [6, 7], and we recently reported that mutant SOD1 knockdown in the cortex of the SOD1G93A rat model of ALS significantly delays disease onset and extends survival [8]. Collectively, this indicates that brain and upper motor neuron dysfunction may contribute significantly to events that lead to motor neuron death in the brain and spinal cord and consequent motor circuitry breakdown in ALS.

Most current strategies using stem cells to treat ALS are focused on the spinal cord. As replacing existing motor neurons is difficult and dysfunctional astrocytes likely contribute to motor neuron death in ALS [9–15], the rational for most of these studies is based on astrocyte replacement, local neuron supplementation or growth factor release [16–20]. Many of these reports show promising results and have led to two human clinical trials...
focusing on the spinal cord as the target [21]. Glial cell line-
derived neurotrophic factor (GDNF) is a powerful growth factor
that protects motor neurons, preserves neuromuscular junc-
tions, and provides beneficial functional effects in ALS rodents,
depending on delivery strategy [22–32]. We have previously
shown that cortical-derived human neural progenitor cells engi-
nereed to secrete GDNF (hNPCGDNF) can survive, differentiate
and release GDNF, and significantly enhance motor neuron sur-



Behavioral Assessment
The Basso, Beattie, and Bresnahan (BBB) locomotor rating scale
[34] is an open field locomotor test of limb function, which is
commonly used to assess the degree of limb paralysis in SOD1G93A rats. The 21-point BBB scale quantifies the ability of
an animal to walk around its environment, with a score of 21
indicating parallel paw placement, coordinated limb movement,
and consistent toe clearance and a score of zero indicating com-
plete paralysis. BBB scores provide an indication of when paral-
ysis begins in any limb and the degree of progression contin-
uing until the animal’s endpoint, which is expected for
this colony to be about 160–190 days of age. BBB scores and
body weights were assessed once or twice weekly by an
observer blinded for genotype and treatment, starting at
approximately 100 days and continuing until disease endpoint.
Scores were recorded from both left and right limbs and the
average for each (hind/fore) limb was taken for the rat’s overall
respective limb score. Disease onset was classified as when an
animal displayed a BBB score of 15 or lower with consequent
progressive decline. In the timepoint experiment, rats were
euthanized earlier (day ~165), when behavior was still signifi-
cantly different between groups and hence when anatomical
state may display a difference. At endpoint the animal will have
lost 20%–30% of its body weight and have a BBB score at or
below 5 in at least one limb. Due to the lack of target-injections
of hNPCs to the neck and trunk region, rats showing neck/trunk
onset were excluded (in both transplanted and noninjected
groups) from behavioral analyses (endpoint study exclusions:



www.StemCells.com © 2018 The Authors Stem Cells published by Wiley Periodicals, Inc. on behalf of AlphaMed Press
incubation (1 : 200, Jackson Labs; West Grove, PA) using an avidin–biotin method was followed by DAB (Vectors Laboratories Inc., Burlingame, Ca) development with nickel ammonium sulfate enhancement.

**Graft Analysis**

To determine the percentage of SC121+ human cells that were GFAP+ and Nestin+ at endpoint, three different fields of view selected at random within the targeted injection region, in three sections, 30 μm apart per rat, were averaged and results were presented as the average of 3 hNPC_GDNF- injected SOD1G93A rats.

**Corticospinal Motor Neuron Analysis**

Corticospinal motor neurons were identified by staining sections for COUP-TF-interacting protein 2 (CTIP2; 1:250; Abcam), which stains the nuclei of motor neurons in layer V along with fluorescent Nissl (Neurotrace; Life Technologies, Waltham, MA) to stain cell bodies. CTIP2-expressing (CTIP2+) cells were counted by performing stereological analysis on three 30-μm sections at 720 μm apart, starting at 1mm posterior to bregma. A standard size rectangular contour, encompassing layer V of the cortex (dimensions, 300 μm height × 1,400 μm width) was drawn for each section. The optical fractionator method was used, where a counting frame of 100 × 100 μm with grid size of 200 × 200 μm (yielding 14–18 counting sites per section) and Gunderson coefficient of error <0.08. CTIP2+ cells were counted and cell bodies were traced to measure cell size using Neurotrace (fluorescent Nissl) staining and the nucleator method (MBF Bioscience software).

**Spinal Motor Neuron Analysis**

Spinal cord sections underwent immunostaining for spinal motor neurons using an antibody against choline acetyltransferase (ChAT) (goat, 1:250, Millipore: Billerica, MA) and counterstained with 4’,6-diamidino-2-phenylindole (DAPI). One ×20 image stack per section (8 sections total, 360 μm apart) was captured to encompass the lateral ventral horn of the lumbar (L3-L5) spinal cord. ChAT+ cells were counted and cell body size was measured using Image J software.

**Nonhuman Primates**

This work was performed under Institutional Animal Care and Use Committee and the Guide for the Care and Use of Laboratory Animals approved protocol at an AAALAC-accredited CRO (Valley Biosystems, Sacramento, CA), in collaboration with Dr. Krystof Bankiewicz (UCSF) who has extensive experience in stereotactic surgery in NHP.

**Cell Transplantation**

hNPC_GDNF were injected under direct visualization into 12 sites, 2 mm apart, unilaterally, into the motor cortex at 10 μl per injection at 50k/μl for a total of 500,000 cells per site. The animal survived surgery, showed no adverse effects, and was euthanized at 30 days post-surgery. Cortical tissue was processed at 40 μm and was shipped to the Svendsen laboratory for immunohistochemical evaluation of hNPC_GDNF survival using an antibody specific to human cytoplasm and GDNF expression. Initial observations of the surface of the brain suggested that cells were targeted to the cortex slightly behind the motor strip.

**Statistical Analysis**

Statistical analyses were performed using Graph Pad Prism software (San Diego, CA). Student’s unpaired t tests, and one-way and two-way ANOVA using Bonferroni post hoc analyses were performed to determine standard error of the mean (S.E.M) with a 95% confidence level. Kaplan Meier survival curve analyses were performed using the Log Rank Test and comparisons of median disease durations and survival times were analyzed by the Wilcoxon Signed Rank Test.

**RESULTS**

**hNPC_GDNF Survive and Release GDNF Following Transplantation into the SOD1G93A Rat Motor Cortex**

We first characterized bilateral injections of hNPC_GDNF throughout the SOD1G93A rat motor cortex that were administered at approximately 80 days of age. Cells delivered to 20 total sites (20k cells per site for a total of 400k cells) survived until rat endpoint (median age 184) in 9 of the 13 transplanted rats, based on the expression of human cytoplasmic marker SC121 (Fig. 1A). Assessed at endpoint, these cells differentiated primarily into glial fibrillary acidic protein (GFAP)+ astrocytes (59.5% ± 4.1% SC121+ cells co-expressed GFAP). Immunostaining for both endogenous and human-specific (SC123 antibody) GFAP showed clear grafts of GFAP+ human cells targeted to cortical layer V, whereas a smaller percentage of SC121+ cells expressed Nestin (20.6% ± 2.6% SC121+ cells expressed Nestin, Fig. 1B). Importantly, immunostaining for GDNF showed that hNPC_GDNF released high levels of GDNF bilaterally, throughout the rostral-caudal axis of the motor cortex (Fig. 1C) in regions expected to affect motor neurons that control forelimb and hindlimb function (Fig. 1D). Interestingly, while GDNF was observed in areas where SC121+ cells are located, GDNF does not always colocalize with these transplanted cells as only approximately 50% of cells are transduced during lentiviral infection [36]. hNPC_GDNF were observed in close proximity to layer V CTIP2 + corticospinal motor neurons (Fig. 1E) and there was efficient uptake of GDNF in some of these host cortical cells (Fig. 1E-G), supporting its function as an actively used trophic factor within injected areas. There was no GDNF release or uptake present in rats transplanted with non-GDNF expressing WT hNPC (hNPC_WT, Fig. 1H).

**hNPC_GDNF Transplantation in the Motor Cortex Results in Delayed Disease Onset and Extended Survival in the SOD1G93A Rat Model of ALS**

Our previous studies injecting hNPC_GDNF into the SOD1G93A rat lumbar spinal cord led to motor neuron preservation but had no effect on disease onset or survival. In contrast, when we knocked down toxic mutant SOD1 in SOD1G93A rat motor cortex, disease onset was delayed and survival was extended [8]. Therefore, we wanted to assess whether the transplantation of hNPC_GDNF into the motor cortex of pre-symptomatic SOD1G93A rats would ameliorate disease pathology. Following hNPC_GDNF injections throughout the motor cortex at pre-symptomatic p80, behavior was monitored throughout the study until euthanasia (Fig. 2A). Using the BBB scale for the assessment of limb paralysis, SOD1G93A rats transplanted with hNPC_GDNF showed significantly enhanced motor function, compared with noninjected control rats (Fig. 2B). This resulted in a 16-day delay
in onset of forelimb paralysis for SOD1\textsuperscript{G93A} rats receiving hNPC\textsuperscript{GDNF} relative to noninjected control SOD1\textsuperscript{G93A} rats (median onset 175 days hNPC\textsuperscript{GDNF} vs. 159 days noninjected: \( p < .05 \), Fig. 2C). Critically, SOD1\textsuperscript{G93A} rats receiving hNPC\textsuperscript{GDNF} also had a significant 14-day extension in survival relative to noninjected control SOD1\textsuperscript{G93A} rats (median survival 184 days hNPC\textsuperscript{GDNF} versus 170 days noninjected: \( p < .05 \), Fig. 2D).

To assess whether GDNF was required in the motor cortex for the functional effects seen here, we added an additional control cohort of animals and repeated the experiment. In this subsequent study, animals were transplanted with either non-GDNF expressing NPCs (hNPC\textsuperscript{WT}) or hNPC\textsuperscript{GDNF} into the motor cortex. Injection of hNPC\textsuperscript{WT} on their own did not lead to improvements in motor function (Fig. 2E). Importantly, this study reproduced the previously observed functional benefit of transplanting hNPC\textsuperscript{GDNF} into the SOD1\textsuperscript{G93A} rat cortex, with significantly improved forelimb BBB scores over time in hNPC\textsuperscript{GDNF}-injected rats relative to noninjected controls and hNPC\textsuperscript{WT}-injected rats. Assessing hindlimb BBB scores in this experiment showed that this function was also significantly improved in SOD1\textsuperscript{G93A} rats receiving hNPC\textsuperscript{GDNF}, relative to those receiving hNPC\textsuperscript{WT} and noninjected controls (Fig. 2F). These results demonstrate that the functional effects observed in the previous experiment were not due to the hNPCs themselves, but rather required both the cells and GDNF. For this study, rats were euthanized at a time point in which motor function was significantly better in hNPC\textsuperscript{GDNF}-injected rats in order to next determine whether this corresponded to enhanced motor neuron survival.

hNPC\textsuperscript{GDNF} Transplantation in the Motor Cortex Results in Improved Health of Large Corticospinal Motor Neurons in SOD1\textsuperscript{G93A} Rats

Corticospinal motor neurons exist in layer V of the motor cortex and express the CTIP2 protein [37]. We have previously reported that corticospinal motor neurons do not die until late in the disease time course in SOD1 rats, whereby at endpoint we observed...
an approximate 25% loss in CTIP2<sup>+</sup> cells >300 \( \mu \text{m}^2 \) relative to WT [8]. To determine whether the presymptomatic transplantation of hNPC<sub>GDNF</sub> in the motor cortex resulted in enhanced corticospinal motor neuron health, we quantified the number of large CTIP2<sup>+</sup> cells present within a defined region in cortical layer V. Fluorescent Nissl was used to stain and visualize cell size, in combination with stereological analysis to calculate the number of large (>300 \( \mu \text{m}^2 \)) CTIP2<sup>+</sup> cells (Fig. 3A). We found that there was no significant difference in the number of CTIP2<sup>+</sup> cells with a size greater than 300 \( \mu \text{m}^2 \) in rats euthanized at time point or endpoint following injections of hNPC<sub>GDNF</sub>, relative to noninjected controls (Fig. 3B). Further analysis, however, revealed that hNPC<sub>GDNF</sub> injections lead to increased numbers of the largest CTIP2<sup>+</sup> cells (>500 \( \mu \text{m}^2 \), Fig. 3C) in both time point and endpoint rats treated with hNPC<sub>GDNF</sub>, relative to noninjected controls euthanized at similar times. This would suggest that atrophy of corticospinal motor neurons, occurring in the SOD1 motor cortex is slowed by the transplantation of hNPC<sub>GDNF</sub>. hNPC<sub>GDNF</sub> Transplantation in the Motor Cortex Results in Enhanced Spinal Motor Neuron Survival in SOD1<sub>G93A</sub> Rats

We next performed ChAT immunostaining in the spinal cord to determine whether spinal motor neurons were protected by hNPC<sub>GDNF</sub> injection into the SOD1<sub>G93A</sub> rat cortex, as a possible mechanism for the observed delayed disease onset and concomitant extended lifespan. In our previous studies, relative to WT rats, SOD1 rats exhibited an early and progressive loss of spinal motor neurons that preceded the loss of corticospinal motor neurons [8]. Starting at 120 days of age, SOD1 rats had already lost 23% of their large spinal motor neurons and by endpoint, these cells were depleted by ~89% relative to WT [8]. In the current experiments, histological analysis (Fig. 3D) demonstrated that the total numbers of ChAT<sup>+</sup> motor neurons and large (>700 \( \mu \text{m}^2 \)) cells as well as the average cell size were similar in both hNPC<sub>GDNF</sub>-injected and non-injected groups at endpoint (day ~185, Fig. 3E-G). This was not surprising given that at endpoint, all animals are paralyzed and presumed to be in a similar anatomical state. In contrast, in the time point experiment, rats were euthanized earlier (day ~165), when behavior was still significantly different between groups and hence when anatomical state may display a difference. As expected, there was a significant increase in total ChAT<sup>+</sup> cells, large ChAT<sup>+</sup> cells and average ChAT<sup>+</sup> cell size in timepoint rats relative to endpoint rats in both non-injected and hNPC<sub>GDNF</sub>-injected groups (*p < .05 at 168 days vs. endpoint, Fig. 4E–4G), confirming the progressive degeneration of spinal motor neurons over time. At the earlier timepoint, rats receiving hNPC<sub>GDNF</sub> trended toward showing a significant increase in total number of spinal motor neurons relative noninjected controls.
However, critically, hNPCGDNF-injected rats showed a significant increase in the number of large ChAT$^+$ spinal motor neurons relative to non-injected controls (Fig. 4E), and ChAT$^+$ spinal motor neuron average cell size was significantly greater by ~25% (Fig. 4G).

While hNPCs and GDNF were clearly present in the cortex, neither were detected in the spinal cord of hNPCGDNF and hNPCWT-injected rats based on histological analysis (data not shown). This indicates that injected cells did not migrate long distances. Thus, motor neurons in the spinal cord were likely
not directly supported by hNPC\textsuperscript{GDNF}, but rather were protected by therapeutically targeting diseased cortical neurons. Given the obvious GDNF uptake by neurons in the motor cortex (Fig. 1), it is possible that low levels of GDNF were anterogradely transported to lower motor neurons but not detectable by histological methods.

hNPC\textsuperscript{GDNF} Survive and Release GDNF in the Cynomolgus Macaque Cortex

To test whether the cells could also be safely delivered to the cortex of larger animals, cell survival and general safety of hNPC\textsuperscript{GDNF} delivery was evaluated following implantation into the nonhuman primate cortex. Following the injection of \(~500,000\) cells per site in 12 sites, the cynomolgus macaque survived surgery and showed no adverse effects with regard to behavior over the next 30 days. Critically, there was pronounced cell survival (Fig. 4A) and robust GDNF secretion (Fig. 4B), which appeared to be taken up by surrounding host cortical cells. These findings show that hNPC\textsuperscript{GDNF} can be transplanted into the cortex of nonhuman primates, migrate away from the transplant site and release GDNF, thus showing safety and facilitating future studies in humans.

**DISCUSSION**

One option to treat ALS is genetic knockdown to reduce toxic mutant protein levels [8, 38]. However, this is primarily relevant to familial ALS patients. Using combined stem cell and gene therapy in order to protect dysfunctional cells is more relevant for both familial and sporadic ALS cases, a critical factor given that 90% of all patients are sporadic, with no known genetic mutation [39]. GDNF-expressing hNPCs, that differentiate in vivo into astrocytes, can protect motor neurons following transplantation in the SOD1\textsuperscript{G93A} rat lumbar spinal cord, but interestingly have no effect on paralysis [23, 25]. Based on these findings, along with our recent demonstration that cortical motor neurons are critically involved in the initiation of ALS [8], we postulated that these same cells transplanted pre-symptomatically into the SOD1\textsuperscript{G93A} rat lumbar spinal cord would have an effect on disease. Here, we show that WT hNPCs alone injected into the motor cortex of SOD1\textsuperscript{G93A} rats had no functional effect. However, hNPCs expressing GDNF improve the health of upper motor neurons, enhance lower motor neuron survival and surprisingly, in contrast to their effects when transplanted to the spinal cord, can also delay paralysis and extend lifespan in the animals.

While studies such as these in transgenic ALS models have incrementally increased our understanding of the disease, there is no preclinical strategy that completely halts or cures the disease. It is now clear that multiple cell types are involved in ALS pathogenesis including microglia, oligodendrocytes, and astrocytes in addition to motor neurons [10, 15, 40–46]. As such, there are a wide range of factors that likely contribute to the disease but that are not completely understood including non-cell-autonomous effects, neuronal excitotoxicity, neuroinflammation, mitochondrial dysfunction, axonal transport defects, protein misfolding/aggregation, oxidative damage, neurotrophin depletion, effects from extracellular mutant SOD1, and aberrant RNA processing [47–56]. Previously, we showed one of the greatest reported delays in disease onset and survival extension in the SOD1\textsuperscript{G93A} rat model by reducing levels of mutant SOD1 in the motor cortex [8]. Knockdown of mutant SOD1 was not specific to motor neurons and was observed throughout the motor cortex, likely affecting toxic protein levels in multiple cell types. Interestingly, one of the largest extensions in survival observed in the SOD1\textsuperscript{G93A} mouse model of ALS resulted from co-targeting independent pathological mechanisms in different cell types in combination [57].

In terms of GDNF, we reported previously that general over-expression administered by early systemic injection of AAV9-GDNF had only modest effects on ALS disease progression in the SOD1 rat [58]. Clearly the appropriate delivery method, tissue targeting, and targeting of specific cell types within the tissue are critical for maximizing the potential for GDNF-mediated therapeutic benefit. We have previously shown that hNPCs genetically engineered to over-express GDNF and then transplanted into the lumbar spinal cord to become astrocytes were able to protect motor neurons in the SOD1 rat [23, 25], and this also occurs following nerve anotomy [59]. There is evidence for GDNF being a trophic factor, working through activation of the receptor and downstream signaling, for several neuronal populations involved in motor control including the corticospinal motor neuron population [30–32, 60–62]. However, providing GDNF alone does not appear to be the most effective approach. This, together with our data showing that hNPCs on their own did not provide benefits, this suggests that there is likely a synergistic/additive effect when providing both healthy support cells and a trophic factor. In light of all of these studies, it is possible that our combined stem cell and gene therapy approach, which provides healthy astrocytes along with a trophic factor to support sick motor neurons (and other cells), could in fact even be combined with additional gene therapy approaches to knock down levels of ALS-related pathogenic proteins such as SOD1 or ataxin2 [63–66].

While it is always a concern that new drugs or therapies might hasten disease progression, in the case of ALS in which
patients typically die within 3–5 years of diagnosis, there is a sense of urgency to bring promising approaches to the clinic. We have now highlighted the importance of the motor cortex in ALS pathogenesis by taking two separate approaches. In this case, hNPC\textsubscript{GDNF} have been shown to promote the health of both upper and lower motor neurons, leading to improved functional outcomes. Critically, these cells have been deemed safe when transplanted in the spinal cord in multiple preclinical models [23, 24, 67]. Specifically, they survive and stably secrete GDNF for up to 7.5 months without tumor formation in the nude rat spinal cord [67] and have been FDA-approved for use in humans. In addition, we have previously shown that hNPC\textsubscript{GDNF} can survive transplantation and release GDNF in the striatum of nonhuman primates and reduce symptoms in a parkinsonian model [68, 69]. But, to our knowledge, this is the first study to show transplantation of neural cells directly into the cortex of these animals. We confirmed that multiple cell injections were safe and that cells survive and secrete GDNF, which is taken up by host cells. We expect that these approaches will be readily translatable to human patients and have a clinical trial currently using these cells but targeting the spinal cord (ClinicalTrials.gov Identifier: NCT02943850). Furthermore, it is possible that maximum benefits in the clinical setting will be observed by treating multiple regions (brain/spinal cord/muscle) in combination. Additionally, considering the homunculus to identify specific regions of the human motor cortex will be important for appropriate targeting and for establishing outcome measures dependent on the location of cell transplant. In order to advance this cortical strategy to the clinic, we are currently performing the required GMP pre-clinical small and large animal studies for cortical administration in future human trials and thus far, these cells have shown no adverse effects in the motor cortex of rats.

There was a correlation between the survival of cells and GDNF expression at endpoint, suggesting that surviving cells still secrete GDNF; otherwise we should see frequent hNPC\textsubscript{GDNF} in the absence of GDNF expression. Although hNPC\textsubscript{GDNF} have a significant beneficial effect on function and survival in SOD1\textsuperscript{G93A} rats, they still reach endpoint due to paralysis. While we found that GDNF expression was closely related to the appearance and location of engrafted human cells, rats with the longest survival did not necessarily have the greatest graft survival/GDNF expression at the time of euthanasia. A limitation here is that it is not possible to determine extent or timing of hNPC\textsubscript{GDNF} cell death and whether an immune response might have diminished the effectiveness of these injected cells. Furthermore, the ALS-related toxic cortical environment might ultimately compromise the supportive effect of hNPC\textsubscript{GDNF}.

There have already been a number of clinical trials transplanting mesenchymal stem cells (MSCs) or neural stem cells not engineered to secrete GDNF into the spinal cord [19, 21, 70–72]. However, none of these trials have had a significant effect on disease progression. The natural secretion of trophic factors including GDNF, as well as the immunomodulatory properties of MSCs [73, 74] has been linked to enhanced motor neuron survival and functional improvements following administration to preclinical animal models of ALS [75–77]. This has made them a promising therapeutic candidate. [19, 78]. However, these cells typically only survive for a matter of weeks and do not produce long-term support cells within the host central nervous system [79–81]. This is in direct contrast to the current study using fetal neural progenitor cells that can differentiate into functional astrocytes, survive long-term, migrate within the spinal cord and release high levels of GDNF.

Enhanced spinal motor neuron survival following hNPC\textsubscript{GDNF} delivery to the spinal cord of SOD1\textsuperscript{G93A} rats [23, 25], along with pre-IND safety and efficacy studies in rodents and mini-pigs, have led to the first-ever cell and gene therapy clinical trial for ALS, in which clinical-grade hNPC\textsubscript{GDNF} are delivered to the spinal cord of ALS patients (ClinicalTrials.gov Identifier: NCT02943850). However, it was clear from our preclinical studies that while targeting the SOD1\textsuperscript{G93A} rat spinal with astrocytes secreting GDNF had a dramatic and significant effect on motor neuron survival, it did not result in delayed disease onset or enhanced survival of the animals. This lack of effect of human cells on function following spinal cord transplantation has been seen by other groups [82] and may be connected with the slow maturation of human cells versus the fast maturation of rodent cells which do show disease progression in rodent models [83]. However, these spinal cord transplantation studies did not consider that the lack of effect may be due to the degeneration and dysfunction of cortical motor neurons. Therefore, while initiating the current safety trial in patients, we are simultaneously pursuing the added therapeutic benefit of targeting both the cervical spinal cord and motor cortex with the same cells, with IND-enabling studies currently underway in both rats and nonhuman primates.

**CONCLUSION**

In conclusion, the fact that human neural progenitor cells releasing GDNF in the ALS rat cortex can both protect downstream motor neurons and, critically, affect disease onset and lifespan suggests a promising therapeutic strategy for this currently untreatable devastating disease.

**ACKNOWLEDGMENTS**

We would like to express our gratitude to Dr. Soshana Svendsen for her critical review and editing of the manuscript. We would like to thank Brandon Shelley and Amanda Hurley for the production and maintenance of hNPCs used in these studies. We would also like to acknowledge Leslie Garcia for immunohistochemistry performed on cytomolgous macaque tissue. We would like to thank Dr. Jerusha Naidoo, Dr. Lluis Samaranch, and John Bringas for assistance with NHP surgery. This work was funded by the ALS Finding a Cure\textsuperscript{+}, a program of The Leandro P. Rizzuto Foundation, The ALS Association, and Department of Defense (Award W81XWH-14-1-0189).

**AUTHOR CONTRIBUTIONS**

G.T.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; P.A. and K.B.: conception and design, collection and assembly of data, final approval of manuscript; A.M., M.A., N.C., L.W., J.-P.V., M.G., P.S., and O.S.: collection and assembly of data, final approval of manuscript; C.S.: conception and design, manuscript writing, final approval of manuscript.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicated no potential conflicts of interest.
REFERENCES

1. Charcot J. De la sclérose latérale amyotrophique. Prog Med 1874;2:341–453.

2. Nagai M, Aoki M, Miyoshi I et al. Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: Associated mutations develop motor neuron disease. J Neurosci 2001;21:9246–9254.

3. Jara JH, Villa SR, Khan NA et al. AAV2 mediated retrograde transduction of corticospinal motor neurons reveals initial and selective apical dendrite degeneration in ALS. Neurobiol Dis 2012;47:174–183.

4. Ozdinler PH, Benn S, Yamamoto TH et al. Corticospinal motor neurons and related subcerebral projection neurons undergo early and specific neurodegeneration in hSOD1G93A transgenic ALS mice. J Neurosci 2011;31:4166–4177.

5. Saba L, Visconi MT, Caioli S et al. Altered functionality, morphology, and vesicular glutamate transporter expression of cortical motor neurons from a presymptomatic mouse model of amyotrophic lateral sclerosis. Cereb Cortex 2015;25:1512–1528.

6. Bae JS, Simon NG, Menon P et al. The puzzling case of hyperexcitability in amyotrophic lateral sclerosis. J Clin Neurorad 2013;9:65–74.

7. Vucic S, Nicholson GA, Kiernan MC. Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain 2008;131:1540–1550.

8. Thomsen GM, Gowing G, Leter J et al. Delayed disease onset and extended survival in the SOD1G93A rat model of amyotrophic lateral sclerosis: Subcortical motor neuron role of suppression of mutant SOD1 in the motor cortex. J Neurosci 2014;34:15587–15600.

9. Boilee S, Yamanaka K, Lobsiger CS et al. Onset and progression in inherited ALS determined by motor neurons and microglia. Science 2006;312:1389–1392.

10. Clement AM, Nguyen MD, Roberts EA et al. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 2003;302:113–117.

11. Yamanaka K, Boilee S, Roberts EA et al. Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. Proc Natl Acad Sci USA 2008;105:7594–7599.

12. Yamanaka K, Chun SJ, Boilee S et al. Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. Nat Neurosci 2008;11:251–253.

13. Wang L, Sharma K, Grisotti G et al. The effect of mutant SOD1 dismutase activity on non-cell autonomous degeneration in familial amyotrophic lateral sclerosis. Neurobiol Dis 2009;35:234–240.

14. Ilieva H, Polyenimendou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol 2009;187:761–772.

15. Kang SH, Li Y, Fukaya M et al. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. Nat Neurosci 2013;16:571–579.

16. Suzuki M, Svendsen CN. Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. Trends Neurosci 2008;31:192–198.

17. Xu L, Shen P, Hazel T et al. Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. Neurosci Lett 2011;494:222–226.

18. Xu L, Yan J, Chen D et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD1 transgenic rats. Transplantation 2006;82:865–875.

19. Thomsen GM, Gowing G, Svendsen S et al. The past, present and future of stem cell clinical trials for ALS. Exp Neurol 2014;262:127–137.

20. Gowing G, Svendsen S, Svendsen CN. Ex vivo gene therapy for the treatment of neurological disorders. Proc Brain Res 2017;230:99–132.

21. Glass JD, Hertzberg VS, Boulis NM et al. Transplantation of spinal cord-derived neural stem cells for ALS: Analysis of phase 1 and 2 trials. Neurology 2016;87:392–400.

22. Suzuki M, McHugh J, Tork C et al. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. Mol Ther 2008;16:2002–2010.

23. Suzuki M, McHugh J, Tork C et al. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. PLoS One 2007;2:e689.

24. Nichols NL, Gowing G, Satriotomo I et al. Intermittent hypoxia and stem cell implants preserve breathing capacity in a rodent model of amyotrophic lateral sclerosis. Am J Respir Crit Care Med 2013;187:535–542.

25. Klein SM, Behrostock S, McHugh J et al. GDNF delivery using human neural progenitor cells in a rat model of ALS. Hum Gene Ther 2005;16:509–521.

26. Wang LJ, Lu YY, Muramatsu S et al. Neurprotection effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 2002;22:6920–6928.

27. Lu YY, Wang LJ, Muramatsu S et al. Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal motoneurons by retrograde transport. Neurosci Res 2003;45:33–40.

28. Asacdi G, Anguelov RA, Yang H et al. Delayed disease onset and extended survival of SOD1 mutant transgenic rats. Proc Natl Acad Sci USA 2006;82:865–875.

29. Anguelov RA, Yang H et al. Transgenic rat model of SOD1 mutant toxicity in neuronal subcerebral projection neurons accelerates onset of disease in ALS mice. Proc Natl Acad Sci USA 2006;82:865–875.

30. Suzuki M, McHugh J, Tork C et al. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. Mol Ther 2008;16:2002–2010.

31. Klein SM, Behrostock S, McHugh J et al. GDNF delivery using human neural progenitor cells in a rat model of ALS. Hum Gene Ther 2005;16:509–521.

32. Wang LJ, Lu YY, Muramatsu S et al. Neurprotection effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 2002;22:6920–6928.

33. Lu YY, Wang LJ, Muramatsu S et al. Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal motoneurons by retrograde transport. Neurosci Res 2003;45:33–40.

34. Asacdi G, Anguelov RA, Yang H et al. Delayed disease onset and extended survival of SOD1 mutant transgenic rats. Proc Natl Acad Sci USA 2006;82:865–875.

35. Fonoff ET, Pereira JF Jr., Camargo LV et al. Functional mapping of the motor cortex of the rat using transudral electrical stimulation. Behav Brain Res 2009;202:138–141.

36. Capowski EE, Schneider BL, Ebert AD et al. Lentiviral vector-mediated genetic modification of human neural progenitor cells for ex vivo gene therapy. J Neurosci Methods 2007;163:338–349.

37. Arlotta P, Molyneaux BJ, Chen J et al. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 2005;45:207–221.

38. van Zundert B, Brown RH Jr. Silencing strategies for therapy of SOD1-mediated ALS. Neurosci Lett 2016;636:32–39.

39. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med 2001;344:1688–1700.

40. Di Giorgio FP, Boulingt GL, Bobrowicz S et al. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. Cell Stem Cell 2008;3:637–648.

41. Haidet-Phillips AM, Hester ME, Miranda CJ et al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol 2011;29:824–828.

42. Sargsyan SA, Blackburn DJ, Barber SC et al. A comparison of in vitro properties of resting SOD1 transgenic microglia reveals evidence of reduced neuroprotective function. BMC Neurosci 2011;12:91.

43. Frakes AE, Ferraiuolo L, Haidet-Phillips AM et al. Microglia induce motor neuron death via the classical NF-kappaB pathway in amyotrophic lateral sclerosis. Neuron 2014;81:1009–1023.

44. Re DB, Le Verche V, Yu C et al. Necrosis drives motor neuron death in models of both sporadic and familial ALS. Neuron 2014;81:1001–1008.

45. Ferraiuolo L, Meyer K, Sherwood TW et al. Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism. Proc Natl Acad Sci USA 2016;113:E6496–E6505.

46. Phillips T, Bento-Abreu A, Nonneman A et al. Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis. Brain 2013;136:471–482.

47. Eisent A, Kim S, Pant B. Amyotrophic lateral sclerosis (ALS): A phylogenetic disease of the corticomotoneuron?. Muscle Nerve 1992;15:219–224.
Glial cell line-derived neurotrophic factor (GDNF) and its receptors - Relevance for disorders of the central nervous system. Neurobiol Dis 2016;97:80–89.

Rakowicz WP, Staples CS, Milbrandt J et al. Glial cell line-derived neurotrophic factor promotes the survival of early postnatal spinal motor neurons in the lateral and medial motor columns in slice culture. J Neurosci 2002;22:3953–3962.

Bosco DA, Morfini G, Karabacak NM et al. -type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci 2010;13:1396–1403.

Forsberg K, Jonsson PA, Andersen PM et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010;5:e11552.

Rakhit R, Robertson J, Vande Velde C et al. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. Nat Med 2007;13:754–759.

Becker LA, Huang B, Bieri G et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature 2017;544:367–371.

Gowing G, Shelley B, Staggenborg K et al. Intracerebral transplantation of bone marrow stromal cells in human bone marrow-derived stromal cells. Cytotherapy 2012;14:56–60.

De Winter F, Vo T, Stam FJ et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010;5:e11552.

Himes BT, Neuhuber B, Coleman C et al. Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. Neurorehabil Neural Repair 2006;20:278–296.

Li Y, Chen J, Wang L et al. Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson’s disease. Neurosci Lett 2001;316:67–70.

Lepore AC, O’Donnell J, Kim AS et al. Human glial-restricted progenitor transplantation into cervical spinal cord of the SOD1 mouse model of ALS. PLoS One 2011;6:e25968.

Lepore AC, Rauck B, Dejea C et al. Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. Nat Neurosci 2008;11:1294–1301.

Kankan R, Vaknin-Dembinsky A, Karussis D. Bone marrow mesenchymal stem cells: Agents of immunomodulation and neuroprotection. Curr Stem Cell Res Ther 2011;6:63–68.

Vercelli A, Mereuta OM, Garbassa D et al. Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. Neurobiol Dis 2008;31:395–405.

Marconi S, Bonacossa M, Scambi I et al. Systemic treatment with adipo-derived mesenchymal stem cells ameliorates clinical and pathological features in the amyotrophic lateral sclerosis murine model. Neuroscience 2010;182:333–343.

Uccelli A, Milanesi M, Principato MC et al. Intravenous mesenchymal stem cell transplantation extends survival and motor function in experimental amyotrophic lateral sclerosis. Mol Med 2012;18:794–804.

Forsberg K, Jonsson PA, Andersen PM et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010;5:e11552.

Rakhit R, Robertson J, Vande Velde C et al. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. Nat Med 2007;13:754–759.

Becker LA, Huang B, Bieri G et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature 2017;544:367–371.

Gowing G, Shelley B, Staggenborg K et al. Intracerebral transplantation of bone marrow stromal cells in human bone marrow-derived stromal cells. Cytotherapy 2012;14:56–60.

De Winter F, Vo T, Stam FJ et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010;5:e11552.

Rakhit R, Robertson J, Vande Velde C et al. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. Nat Med 2007;13:754–759.

Becker LA, Huang B, Bieri G et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature 2017;544:367–371.

Gowing G, Shelley B, Staggenborg K et al. Intracerebral transplantation of bone marrow stromal cells in human bone marrow-derived stromal cells. Cytotherapy 2012;14:56–60.

De Winter F, Vo T, Stam FJ et al. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 2010;5:e11552.

Rakhit R, Robertson J, Vande Velde C et al. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. Nat Med 2007;13:754–759.