

Concise Review: Stem Cell Therapies for Amyotrophic Lateral Sclerosis: Recent Advances and Prospects for the Future

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a lethal disease involving the loss of motor neurons. Although the mechanisms responsible for motor neuron degeneration in ALS remain elusive, the development of stem cell-based therapies for the treatment of ALS has gained widespread support. Here, we review the types of stem cells being considered for therapeutic applications in ALS, and emphasize recent preclinical advances that provide supportive rationale for clinical translation. We also discuss early trials from around the world translating cellular therapies to ALS patients, and offer important considerations for future clinical trial design. Although clinical translation is still in its infancy, and additional insight into the mechanisms underlying therapeutic efficacy and the establishment of long-term safety are required, these studies represent an important first step toward the development of effective cellular therapies for the treatment of ALS. STEM CELLS 2014;32:1099–1109

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a lethal adult-onset neurodegenerative disorder characterized by the loss of both upper and lower motor neurons. Sporadic and familial forms are clinically and pathologically indistinguishable, with symptoms including muscle weakness and atrophy that present in either bulbar muscles and/or in the limbs. In almost all cases, death occurs within 3–5 years of diagnosis when progressive motor neuron degeneration affects the diaphragm and leads to respiratory failure. Riluzole, the only FDA-approved treatment for ALS, extends survival for merely a few months [1], highlighting the need for more effective therapies.

The development of targeted therapies for ALS, however, has been hindered by the fact that the mechanisms responsible for disease onset and progression largely remain unknown. Only 10%–15% of cases of ALS are familial, while the remaining 85%–90% of cases are classified as sporadic. Several genetic mutations are associated with familial ALS, including mutations in $\text{Cu}^{2+}/\text{Zn}^{2+}$ superoxide dismutase (SOD1) and TAR DNA binding protein-43 [2–5]. Most recently, hexanucleotide repeat expansions in the 5' noncoding region of the C9orf72 gene have been identified as the most common cause of familial ALS [6]. Among the many proposed

mechanisms for the more common sporadic form of ALS [4, 7, 8], oxidative stress and glutamate toxicity induce a toxic cellular and spinal cord milieu, respectively, while neurofilament aggregation and axonal transport defects may be associated with altered mitochondrial trafficking and impaired retrograde transport of peripherally derived neurotrophic factors [4, 8–10]. Recent evidence has also linked protein aggregation and impaired RNA processing to ALS pathogenesis [11, 12]. Furthermore, altered peripheral immunological responses and neuroinflammation are emerging as important effectors of the ALS disease course [13, 14]. Non-neuronal cells such as microglia, astrocytes, and oligodendrocytes also contribute to ALS pathogenesis, via alterations to the spinal cord microenvironment, increased glutamate excitotoxicity, and/or impaired neuronal metabolic support [2, 15–17]. In fact, oligodendrocyte dysfunction is evident early in the disease course before symptom onset [18]. Similarly, denervation at neuromuscular junctions and axonal defects precede symptom onset and motor neuron loss, and studies have shown that connectivity along the entire motor axis, from the corticospinal tract to motor neurons and neuromuscular junctions, is compromised in ALS [19–21]. Therefore, treatments that influence multiple pathogenic mechanisms in ALS and that

provide motor neuron support as well as maintain neuronal circuitry are likely required to have the most significant impact on the disease course.

Because of the multifaceted nature of ALS, the emerging concept of stem cell-based therapeutics for ALS treatment has garnered increasing support [22–24]. In this review, we discuss the types of stem cells being considered for therapeutic applications in ALS, highlighting preclinical data supporting the rationale behind the potential efficacy of each cell class and treatment approach. We also discuss some of the early translational studies providing stem cell-based therapies to ALS patients around the world. Although clinical translation is still in its infancy and additional insights into the mechanisms underlying efficacy and the establishment of long-term safety are required, these studies represent an important first step toward the development of cellular therapies for the treatment of ALS.

PRECLINICAL ADVANCES IN STEM CELL THERAPIES FOR ALS

Preclinical *in vitro* and *in vivo* studies have provided tremendous insight into which types of stem cells are likely to offer therapeutic benefits in ALS [22, 23]. These lines vary in their derivation source, differentiation potential, and availability; features that all contribute to the advantages and limitations of each population. Understanding how stem cells may confer benefit is also of utmost importance, as transplanted cells can offer cell replacement, provide support through paracrine effects and growth factor production, or alter the immune response and inflammation through cytokine production. Furthermore, treatments aimed at activating endogenous stem cell niches provide a therapeutic option to enhance natural neuroprotective mechanisms. Thus, determining the desired outcomes of stem cell-based therapies is critical for continued therapeutic development. Finally, therapeutic delivery approaches vary and selection of the optimal strategy to achieve the desired effects on ALS pathogenesis warrants important consideration.

Embryonic Stem Cells

Embryonic stem cells (ESCs) have the ability to differentiate into all germ layers, and serve as a resource for both cellular replacement in ALS and for disease modeling when differentiated into motor neurons. Early studies examining intraspinal transplantation of ESC-derived motor neurons into G93A-SOD1 rats demonstrated transient functional improvements; however, there was no apparent axonal projections to the periphery, no effect on neuromuscular junction formation, no long-term effects on the lifespan of the rats, and limited graft survival [25]. Considering the fact that transplanted cells must project axons over long distances in the context of a toxic spinal cord, these results are not surprising and support the contention that direct motor neuron replacement is unlikely to affect the disease course in ALS [25]. Furthermore, their limited supply as well as the fact that human ESCs are subject to strict regulatory policies has hampered continued ESC-based therapeutic advances for ALS; however, the utilization of ESCs for high-throughput drug screening continues, and prospects for future drug development efforts have already been identified using such strategies [26].

Mesenchymal Stem Cells

The therapeutic development of many other stem cell types for clinical application in ALS, conversely, is gaining momentum. Umbilical cord stem cells (UBCs) are harvested from umbilical cord blood and provide a source of mesenchymal stem cells (MSCs) capable of differentiating into mesenchymal and potentially even neuronal lineages [27]. In both G93A-SOD1 and wobbler mice, intracerebroventricular injection of UBCs attenuated progression, and the treatment improved survival by approximately 10% in G93A-SOD1 mice; however, grafted cells were identified within the ventricles and not the spinal cord, suggesting that the observed effects were mediated by production and release of neuroprotective factors, including anti-inflammatory cytokines and chemokines [28]. Similarly, retro-orbital injection of genetically engineered UBCs expressing increased levels of vascular endothelial growth factor (VEGF) and fibroblast growth factor in G93A-SOD1 mice prompted differentiation to astrocytic lineages that produce protective growth factors to improve the motor neuron microenvironment [29]. Retro-orbital delivery of UBCs improved neuromuscular transmission [30], and intravenous UBC administration delayed disease progression by 15%, induced anti-inflammatory effects, reduced microglial activation, and increased survival up to 20%–25% in G93A-SOD1 mice [31, 32]. Intraspinal transplantation of UBCs at an early stage in the disease course improved motor function, attenuated motor neuron loss, reduced astrogliosis, and improved survival by up to 12 days in female G93A-SOD1 mice [33], further supporting the potential of UBC-based therapies in ALS.

Multiple approaches using bone marrow-derived MSCs are also being developed for clinical translation based on the relative accessibility and abundance of MSCs compared to other stem cell classes and their potential for autologous cellular therapy development. Recent reports of MSC cross-lineage differentiation to produce myoblasts and neurons are intriguing, but must be interpreted with caution as this is a relatively new finding and further study is required [24, 34–36]. Intravenous, intrathecal, intracerebral, and intraspinal delivery of autologous MSCs in G93A-SOD1 mice confer a range of beneficial effects on the disease course, including improved motor function, attenuated motor neuron loss, and prolonged survival [22, 23]. G93A-SOD1 mice receiving intraspinal MSC transplants exhibit favorable effects on neuroinflammation, astrogliosis, and microglial activation [37]. Furthermore, induction of neural differentiation of MSCs via neurogenin 1 expression enhances MSC homing to the CNS following intravenous administration in ALS mice and is associated with delayed disease onset and improved motor function [38].

An alternative strategy, however, given that MSCs do not naturally differentiate into neural lineages, is the use of MSCs as a vehicle to deliver neuroprotective factors to the CNS. Intracerebroventricular injection of G93A-SOD1 mice with MSCs expressing glucagon-like peptide 1, a peptide with antioxidant properties, confers significant effects on the disease course, including a 15-day delayed onset, a 13-day improvement in survival, and attenuated neuroinflammation, astrogliosis, and microglial activation [39]. Intramuscular injection of MSCs expressing increased levels of glial-derived neurotrophic factor (GDNF) improved motor neuron health and extended survival by 28 days in G93A-SOD1 rats [40]. The caveat,

however, with MSCs or any autologous cellular therapy, is that cells harvested from diseased individuals for therapeutic purposes (in this case with ALS) are inherently predisposed to the disease process. This could clearly impact the therapeutic utility of autologous cells. While some studies characterizing the *in vitro* properties of MSCs from both ALS patients and healthy controls demonstrate that there are no distinct differences in cellular properties or neuronal differentiation [34, 41], other studies have indicated that ALS patient MSCs exhibit reduced neurotrophic factor secretion and decreased migration [42], and the degree of these deficits correlates with poorer prognosis in ALS patients [43]. Impaired neuroprotective capacity has been observed in MSCs from adult G93A-SOD1 rats [44], and the stability and cellular properties, including neurotrophic profile and anti-inflammatory potential, of MSCs from ALS patients also differ between passages *in vitro* [45]. Together, these studies suggest that MSCs from healthy donors or utilization of optimally passaged MSCs may confer improved efficacy for cellular therapy development over the use of autologous MSCs from an ALS patient.

Progenitor Cells

Neural progenitor cells (NPCs) are emerging as a promising cellular therapy for clinical translation in ALS. In ALS models, motor neuron degeneration triggers endogenous NPC niches in the CNS to proliferate, migrate, and promote neurogenesis in the spinal cord as a natural response to disease [46, 47]; however, the limited number of endogenous NPCs is likely insufficient to combat the toxic, progressive degeneration associated with ALS. Thus, NPC cell lines with robust growth properties and neurogenic potential have been developed [48, 49] and transplantation of NPCs has been extensively studied as an attempt to augment this natural defense mechanism. NPCs have been delivered via intravenous, intrathecal, and most commonly via intraspinal methods to both G93A-SOD1 mouse and rat models, and the effects on the disease course and cell fate have been extensively characterized [22, 23]. Intraspinal grafting of human NPCs in ALS rats conferred improvements in survival of more than 10 days and protective effects on motor neuron number and motor function that are attributed to the observed ability of transplanted NPCs to integrate into the spinal cord, differentiate, and form functional synapses with host motor neurons [50–52]. Recent observations in G93A-SOD1 rats receiving intraspinal NPC injections, however, revealed that although transient effects on motor neuron number and function were observed in the vicinity of the cellular grafts, electrical recordings of motor-evoked potentials reflected impaired transmission along the descending motor tract and limited effects on survival were present, suggesting that attenuating neuronal loss along the entire corticospinal tract is necessary to achieve meaningful effects on disease progression [21]. This is further supported by studies in G93A-SOD1 rats demonstrating that targeting intraspinal NPC transplants to multiple regions of the spinal cord significantly prolongs survival by 17 days [53]. As in MSC studies, the development of enhanced NPC lines expressing increased levels of growth factors such as GDNF and VEGF also confers improvements in motor neuron number and motor function following transplantation [54, 55], suggesting that combination therapies may warrant consideration in the future. Recent insight into potential crosstalk between trans-

planted NPCs and endogenous progenitor cell niches in the spinal cord supports the possibility that cellular therapy approaches can induce protection by activating endogenous neuronal repair mechanisms as well [56]. Thus, NPC transplantation has the ability to support motor neurons, provide neurotrophic support, enhance endogenous neurorepair mechanisms, and ultimately maintain neurocircuitry and provide meaningful effects on the ALS disease course.

Finally, the multifaceted mechanisms and variety of cell types proposed to contribute to ALS pathogenesis support cellular therapy development using non-neuronal progenitors. Intracerebroventricular injection of skeletal muscle stem cells induces anti-inflammatory cytokine production, improved motor function, and protection of neuromuscular junctions in wobbler mice [57]. Intraspinal transplantation of glial-restricted progenitor cells into the cervical spinal cord of G93A-SOD1 rats, as a means to increase astrocyte numbers in spinal cord regions controlling critical respiratory function, attenuates motor neuron loss, slows respiratory functional declines, and improves survival by approximately 17 days [58]. Olfactory ensheathing stem cells (OESCs), a type of glial cell that assists in axonal regeneration and is currently in use for spinal cord injury, have also demonstrated positive effects on ALS progression in preclinical studies, including evidence of myelination, increased motor neuron numbers, and more than a 6-day increase in survival following intraspinal transplantation in G93A-SOD1 rats [59]. Analysis of oligodendrocytes in ALS patients and G93A-SOD1 mice revealed defects in the maturation and function of newly proliferating progenitors following degeneration of resident oligodendrocytes during the disease course, suggesting that cellular therapies that restore oligodendrocyte function may be beneficial in ALS [18, 60]. In support of this contention, deletion of G93A-SOD1 in oligodendrocytes of ALS mice delayed disease onset and improved survival [18].

Taken together, these and other studies (detailed further in [22, 23, 61]) demonstrate that multiple classes of stem cells have the potential to impact ALS pathogenesis in preclinical settings by improving the motor neuron environment, replacing lost neuronal and non-neuronal cells within the spinal cord, supporting neuromuscular junctions, and/or modulating the immune response (Fig. 1). Additional discernment of how each stem cell type contributes to these effects and how to most effectively target stem cells to achieve these effects will be incredibly important for selecting the appropriate cell type and designing therapeutic delivery approaches for future clinical translation.

TRANSLATING STEM CELL THERAPIES TO ALS PATIENTS

Although the age of stem cell-based therapeutics is just beginning, a handful of cellular therapy trials for ALS have been completed in different countries around the world. These recent trials are summarized in Table 1 and include vast differences in the number of patients, cell type, delivery method, and outcome measurement strategies; however, each study has the potential to contribute to our current understanding of the safety and feasibility of stem cell therapies for ALS, as these variables provide important considerations for future trial design and clinical application.

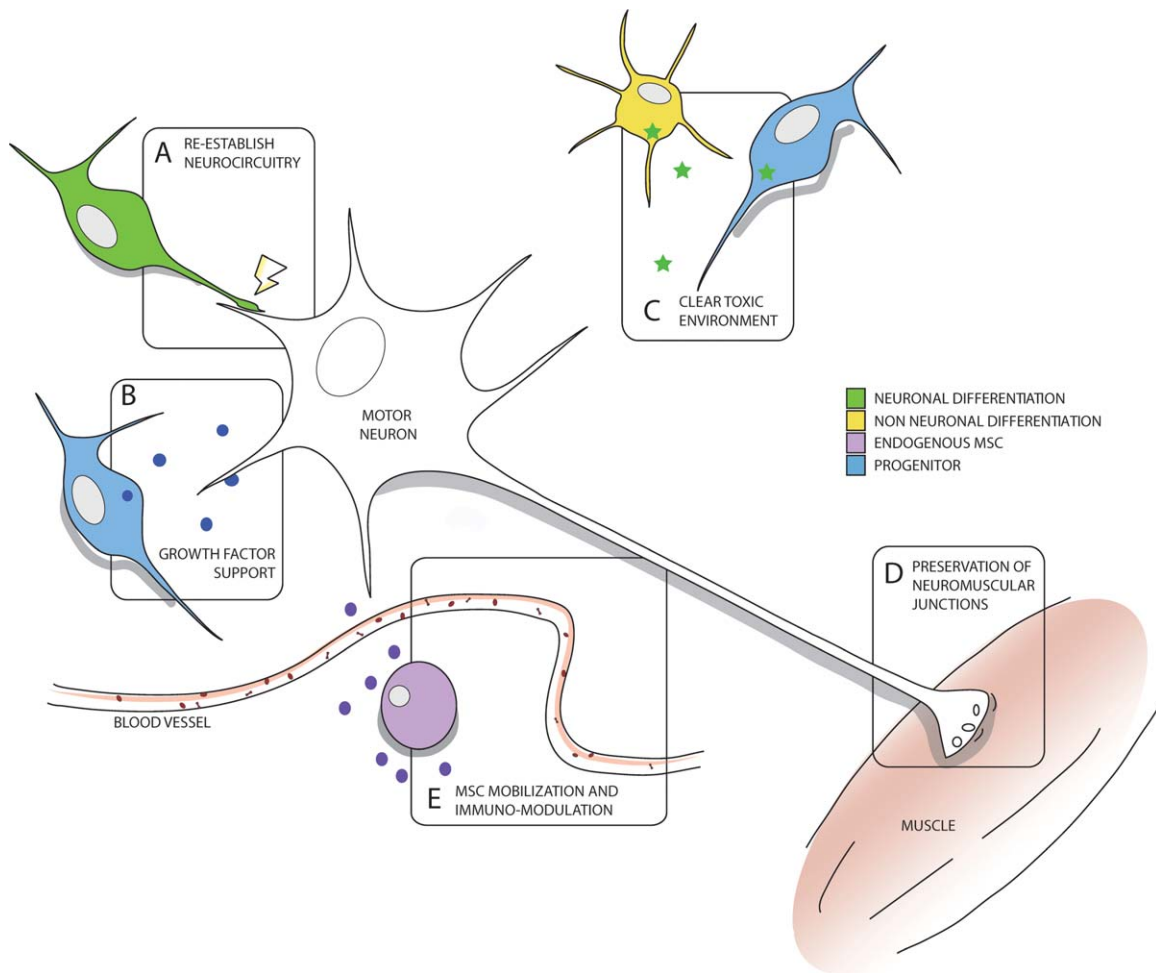


Figure 1. Potential mechanisms of stem cell efficacy in amyotrophic lateral sclerosis. In addition to motor neurons, multiple cell types within the motor neuron microenvironment play a role in disease pathogenesis; therefore, the delivery of stem cell-based therapies (blue) has the potential to provide support through many different mechanisms. Within the spinal cord, stem cells that differentiate into neurons (green) can synapse with existing motor neurons to re-establish or maintain neurocircuitry (A) as well as provide neurotrophic support (B). Differentiation of stem cells into non-neuronal cell types (yellow) within the spinal cord microenvironment can also impact disease progression by providing neurotrophic support (B), and attenuating oligodendrocyte dysfunction and mitigating toxicity (C). In the periphery, stem cell transplantation into muscle can provide critical support to maintain functional neuromuscular junctions (D). Finally, the mobilization of endogenous MSCs from the bone marrow into the circulation can also induce immunomodulatory effects that attenuate inflammatory responses within the spinal cord via the production of cytokines and other anti-inflammatory mediators (E). Abbreviation: MSC, mesenchymal stem cell.

Clinical Application of OESCs

OESC transplantation for ALS has commenced in People's Republic of China based on positive effects, including axonal regeneration, remyelination, and functional improvements, in spinal cord injury studies [81]. In a small controlled study involving 35 ALS patients, 15 of which received OESC transplants and 20 untreated controls, individuals receiving intracranial OESC transplants showed decreased progression, as measured by the ALS functional rating scale (ALSFRS), over the 4-month follow-up period [62]. Based on these and other short-term results from this group [82], over 500 ALS patients have since received OESC transplants in People's Republic of China, with the majority of individuals receiving a single intracranial OESC injection, and 42 patients receiving two to five rounds of OESC injections [63]. Evaluation of the 42 patients receiving multiple rounds of OESCs revealed improvements in ALSFRS and ALS Norris Scale scores as well as improvements

in neurological and pulmonary function after repeated intervention [63]. Independent follow-up case reports on individuals receiving OESC transplants in People's Republic of China, however, do not support the clinical translation of this OESC therapy. Evaluation of seven patients for 1 year revealed no significant objective improvements and two patients experienced serious side effects [83], and similarly, no benefit was seen in an elderly woman who experienced accelerated disease progression and severe side effects following OESC therapy [84]. Furthermore, post-mortem characterization of the cellular grafts in two Italian patients treated in People's Republic of China exhibited evidence of graft encasement, the presence of undifferentiated cells, and no evidence of neurotrophism or regeneration [85]. Thus, while the large Chinese study reports that OESCs may offer benefit in ALS, other reports criticize the observed outcomes and do not support the clinical translation of this therapeutic approach at this time. Furthermore, these findings strongly emphasize the

Table 1. Clinical applications of stem cell therapies in ALS.

| Stem cell type | Delivery method | Country | Patients no. | Desired outcomes | Conclusions | Additional details | Reference(s) |
|-----------------------------|-----------------|----------------------------|------------------------|------------------|---|--|--|
| OESC | Intracranial | People's Republic of China | 15 OESC 20 control | Efficacy | Beneficial effects on disease progression | Follow-up for 4 months; seven patients receiving OESCs showed improvements and two stabilized; only one in the control group remained stable | Huang et al. [62] |
| | Intracranial | People's Republic of China | 42 | Efficacy | Delayed progression and restoration of function | Report on patients receiving two to five treatments (out of 507 total patients receiving cellular therapy) | Chen et al. [63] |
| Endogenous MSC mobilization | n/a | Canada | 8 | Safety | Approach is safe and feasible | Pilot study; G-CSF treatment mobilized MSCs with no adverse effects | Cashman et al. [64] |
| | n/a | Italy | 24 | Safety | Approach is safe and feasible | Endogenous MSC mobilized by four G-CSF stimulation treatments at 3-month intervals; At 1 year, no increase in disease progression at rates, anti-inflammatory response observed | Chio et al. [65] |
| | n/a | Israel | 17 G-CSF 18 Placebo | Efficacy | Approach is safe and feasible; no significant effect on disease progression | Endogenous MSC mobilized by G-CSF stimulation every 3 months for 1 year | Nefussy et al. [66] |
| Autologous MSC | Intraspinal | Turkey | 13 | Safety | Approach is safe and feasible | Cervical (C1-C2) injections; enrolled patients had bulbar symptoms; nine patients demonstrated electromyography improvements and one had stabilized at 1 year | Deda et al. [67] |
| | Intraspinal | Italy | 9 | Safety | Approach is safe and feasible | High thoracic (T7-T9) injections; no apparent toxicity, transplant-related adverse events, or structural changes; evidence of slowed functional decline in four patients; follow-up of 4 and up to 9 years | Mazzini et al. [68] Mazzini et al. [69] |
| | Intraspinal | Italy | 10 | Safety | Approach is safe and feasible | High thoracic (T4-T6) injections; no apparent toxicity, transplant-related adverse events, or structural changes; follow-up of 2 or up to 5 years | Mazzini et al. [70] Mazzini et al. [69] |
| | Intraspinal | Spain | 11 | Safety | Approach is safe and feasible | No acceleration in decline noted; increased motor neuron numbers noted in treated spinal cord segments at autopsy; motor neurons surrounded by CD90+ cells without degenerative ubiquitin deposits | Blanquer et al. [71] |

Table 1. Continued

| Stem cell type | Delivery method | Country | Patients no. | Desired outcomes | Conclusions | Additional details | Reference(s) |
|--|---|-------------|------------------------------|--------------------|--|--|---|
| | Intrathecal | India | 10 | Safety Efficacy | Approach is safe and feasible; initial trend toward stabilization of disease | No significant acceleration of ALSFRS decline after short-term follow-up at 1 year; confirmation of benefit required with long-term follow-up | Prabhakar et al. [72] |
| | Intrathecal or combined intrathecal/intravenous | Israel | 10 intrathecal 9 combined | Safety | Approach is safe and feasible | Both intrathecal and combination intrathecal/intravenous administration approaches are safe; SPIO labeling in a subset of patients revealed cells in meninges, subarachnoid space and spinal cord; immunomodulatory effects observed; follow-up period of 6–25 months with MRI at 1 year | Karussis et al. [73] |
| | Intraventricular | South Korea | 1 | Safety | Approach is safe and feasible | Ommaya reservoir used to administer three injections at 1 month intervals; no conclusions on efficacy due to advanced disease state of patient | Baek et al. [74] |
| | Motor Cortex | Mexico | 10 MSC 10 control | Safety | Approach is safe and feasible | Pilot study; procedure is safe and well-tolerated; survival statistically higher in treated patients | Martinez et al. [75] |
| | Motor Cortex | Mexico | 65 | Safety | Approach is safe and feasible | Targeted frontal motor cortex to improve upper motor neuron function; larger controlled trial required to assess efficacy | Martinez et al. [76] |
| T-cell vaccination coupled with autologous MSC and NPC therapy | Intravenous | Argentina | 7 | Efficacy | Approach is safe and feasible; median survival extended | Dual cellular therapy approach; neurological recovery noted in five patients | Moviglia et al. [77] |
| NPC | Intraspinal | U.S. | 12 | Safety | Approach is safe and feasible | Lumbar (L2-L4) injections; follow-up of 6–18 months; no acceleration of disease course present; one patient demonstrated improvements; continued follow-up also available | Glass et al. [78] Riley et al. [79] Riley et al. [80] |
| | Intraspinal | U.S. | 6 | Safety | Approach is safe and feasible | Cervical (C3-C5) injections; three patients were part of the initial lumbar cohort (above) and received dual targeting to both lumbar and cervical regions | Riley et al. [80] |

Abbreviations: ALSFRS, amyotrophic lateral sclerosis functional rating scale; MSC, mesenchymal stem cell; NPC, neural progenitor cell; OESC, olfactory ensheathing stem cell; SPIO, super paramagnetic iron oxide.

need for continued research, including additional preclinical validation, detailed graft characterization, and long-term well-designed trials, to support the safety and utility of OESC-based therapies in ALS patients.

Clinical Applications of MSC-Based Therapies for ALS

The largest number of cellular therapy trials for ALS involves MSCs. Three clinical trials have tested strategies using granulocyte colony-stimulating factor (G-CSF) to mobilize endogenous MSCs in ALS patients. Trials based in Canada and Italy have demonstrated safety of the approach, confirmed mobilization of MSCs, and demonstrated anti-inflammatory responses in the spinal cord [64, 65]. A pilot study in Israel also demonstrated safety and feasibility of G-CSF-induced MSC mobilization; however, no significant effects on disease progression were noted compared to placebo-treated controls [66]. Alternatively, a number of trials assessing autologous MSC treatment approaches around the world have demonstrated the safety and feasibility of intraspinal, intrathecal, and intracerebral MSC transplants [24]. Although safety was the primary question in the majority of these studies, secondary outcome assessment in a trial of 13 patients in Turkey receiving intraspinal C1-C2 MSC transplants demonstrated encouraging effects on electoneuromyography measures in the majority of patients [67], and secondary outcome evaluation of 11 patients receiving intraspinal MSC transplants in Spain revealed increased motor neuron numbers, reductions in the presence of ubiquitin deposits in motor neurons, and evidence of neurotrophism in treated spinal cord segments [71]. In addition, a controlled pilot study in Mexico examining the safety of intracranial MSC delivery in 20 ALS patients reported that survival was significantly extended in treated patients [75]. Together, these trials provide important insight into the safety and feasibility of autologous MSC-based therapies in ALS patients, although determining the efficacy of these approaches requires continued insight into the ability of MSCs to home to the CNS, insight into the mechanisms of neuroprotection, and large, controlled studies to evaluate efficacy.

Clinical Application of NPC-Based Therapies

Given the vast amount of preclinical support for NPC-based therapies, an FDA-approved clinical trial assessing the safety and feasibility of intraspinal injection of human spinal stem cells (HSSCs) in ALS patients is currently in progress in the U.S. [22, 23, 78, 79]. Using a customized injection device designed for safe, reproducible, accurate delivery of biologics to the spinal cord [22, 78, 79, 86–89], 18 intraspinal transplantation surgeries were performed following a risk escalation paradigm to complete phase I of the trial. Twelve patients received L2-L5 lumbar-targeted intraspinal injections and six patients received C3-C5 cervical-targeted intraspinal injections with no major adverse effects attributed to the surgery or cells [78–80]. Of note, the last three patients receiving cervical HSSC transplants had previously received lumbar transplants, demonstrating that targeting multiple levels of the spinal cord is feasible in ALS patients, an approach associated with improved therapeutic efficacy in preclinical studies [53]. Patients were all evaluated for multiple functional measures, including ALSFRS, respiratory function, and muscle strength, and demonstrated no acceleration in progression fol-

lowing transplants, and one patient exhibited improved functional measures; however, phase II of the trial, which began in September 2013, will be required to assess HSSC dosing and efficacy of the intervention.

THE FUTURE OF STEM CELL THERAPIES FOR ALS

Overall, multiple groups have assessed the safety of cellular grafting along the entire neural axis, using systemic approaches and targeting brain regions as well as the upper and lower spinal cord, and demonstrated that delivery approaches and the introduction of stem cell populations into these regions can be successfully and safely accomplished. While considerable work is still required, these data provide proof-of-concept that cellular grafting as a therapy for ALS is feasible and support a continued focus on refining stem cell-based therapeutic approaches to achieve maximal benefit in ALS.

As we look to the future, a number of important considerations must still be addressed to support stem cell therapies for the treatment of ALS. First, elucidating the proper approach to deliver or target cellular therapies to regions where it will have maximal benefit in ALS patients is of utmost importance. As detailed in the previous section, strategies ranging from intravenous, intraspinal, and intraventricular injection of cellular therapies to treatments designed to activate or mobilize endogenous progenitor populations are currently being pursued. While there may not be a single ideal approach, establishing standardized practices for the delivery of cellular therapies to ensure reproducible injection volumes and targeting accuracy will assist in the design and interpretation of future clinical trials. For example, the recent development and utilization in a clinical trial of novel devices for intraspinal delivery of cellular therapies is already realizing these objectives [22, 78, 79, 86–89].

Second, confirmation of graft survival is imperative to achieve sustained efficacy. In many preclinical *in vivo* studies, the identification of grafted human cells in animal models is facilitated by immunohistochemical identification using human-specific markers [50, 52, 86]. Upon clinical translation, however, more sophisticated techniques to identify and follow the fate of grafted cells will be required. For instance, the labeling of cells with superparamagnetic iron oxide nanoparticles or reporter genes allows cell graft tracking using advanced imaging technologies [28, 57, 90–95]. Notably, these approaches can provide insight into the migratory potential of grafted cells following systemic or targeted injections, as demonstrated in both a large animal and human trials [73, 86]. Understanding how grafted cells migrate into or within the spinal cord will be necessary to optimize delivery approaches and maximally impact critical cellular populations, including both upper and lower motor neurons, and maintain functional neurocircuitry along the entire corticospinal tract.

Third, requirements for immunosuppression must also be determined. Graft survival of transplanted human NPCs is enhanced using combined immunosuppression regimens in ALS models and in a large mammal, the Gottingen minipig [86, 96]. What is now required are data from human trials to determine the role of the immune system and immunosuppression requirements when transplanting human cells into

ALS patients. Additional longitudinal data from ongoing and future clinical trials will provide important insight into graft survival, characterization, and treatment efficacy.

As we continue to move forward and optimize how to best deliver cellular therapies, emphasis on the design of future clinical trials will also be necessary to glean meaningful insight into the safety and efficacy of clinical outcomes [97, 98]. Common outcome measures in these ALS trials include ALSFRS assessment and other functional measures as well as survival; however, the elucidation and inclusion of novel prognostic biomarkers in ALS trials may provide additional power and inform patient selection criteria [97, 98]. Given the heterogeneous presentation and rapid progression of ALS, consideration of limb versus bulbar onset ALS and early versus late disease, and knowledge of disease progression rates prior to cellular delivery, may be required patient selection criteria in future trials.

Finally, novel cellular therapy development may be possible with continued advances in the field of stem cell research. Since the initial reports generating induced pluripotent stem (iPS) cells from somatic tissue, established differentiation protocols have enabled the development of patient-specific iPS-derived motor neurons from ALS patients for in vitro characterization and drug screening [99–102]. These cells may also offer an autologous source for cellular therapy that circumvents the need for immunosuppression; however, the clinical application of iPS cellular therapies has not yet been attempted or realized, and insight into how the inherent predisposition to disease these cells may possess affects their therapeutic potential is required. Nonetheless, constant protocol refinements, such as alternative methods to introduce genetic reprogramming factors and the most recent reports of iPS cell generation using a chemical-based approach, are supporting the potential for future preclinical and clinical therapeutic applications of this technology [103–107]. Continued development of enhanced stem cell lines, such as those expressing increased levels of neurotrophic growth factors, may also gain ground in future translational studies, as this approach has the potential to combine the benefits of growth factor delivery in ALS with cellular support offered by stem cell-based therapies and form a multifaceted attack on ALS [108, 109]. In addition, cellular therapy approaches combining NPC, glial progenitor cell, and/or skeletal muscle cell treatment modalities may offer additive benefit against pathogenic mechanisms conferred at the level of neurons, glia, and in muscle at peripheral neuromuscular junctions. Indications for the future combination of immune modulation with stem cell therapies as a possible therapeutic avenue also exist, as an Argentinian clinical trial combining MSC or NPC transplan-

tation with T-cell vaccination demonstrated safety, improvements in median survival, and evidence of neurological recovery in five out of seven patients [77]. Overall, ensuring that cellular therapies are capable of providing long-term benefits that affect motor neurons, their environment, and connectivity along the entire neuroaxis is likely critical to achieve meaningful outcomes in ALS.

CONCLUSIONS

Although much work remains to be done, the increasing focus on preclinical research for stem cell therapies and the recent translation of a small number of these therapies to clinical trials have set the stage for continued progress. In the near future, efforts must continue to determine the most efficacious cell type and identify appropriate approaches to safely administer cellular therapies to achieve positive outcomes in ALS. With the establishment of best practice guidelines for cellular therapies, it may then be possible for future endeavors to address strategies that use novel cellular sources, engineer enhanced stem cells, or develop combinatorial therapeutic approaches in order to provide potentially meaningful therapies for this lethal disorder.

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AUTHOR CONTRIBUTIONS

J.S.L.: conception and design, collection and/or assembly of data, data analysis and interpretation, and final approval of manuscript; S.A.S.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; E.L.F.: conception and design, financial support, manuscript writing, and final approval of manuscript. J.S.L. and S.A.S. contributed equally to this article.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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