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REVIEW

Recent Advances and the Future of Stem Cell Therapies in Amyotrophic Lateral Sclerosis

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Abstract Amyotrophic lateral sclerosis is a progressive neurodegenerative disease of the motor neurons without a known cure. Based on the possibility of cellular neuroprotection and early preclinical results, stem cells have gained widespread enthusiasm as a potential treatment strategy. Preclinical models demonstrate a protective role of engrafted stem cells and provided the basis for human trials carried out using various types of stem cells, as well as a range of cell delivery methods. To date, no trial has demonstrated a clear therapeutic benefit; however, results remain encouraging and are the basis for ongoing studies. In addition, stem cell technology continues to improve, and induced pluripotent stem cells may offer additional therapeutic options in the future. Improved disease models and clinical trials will be essential in order to validate stem cells as a beneficial therapy.

Key words Amyotrophic lateral sclerosis · Stem cell therapy · Cell transplantation · Neural progenitor cell · Mesenchymal stem cell · Granulocyte-colony stimulating factor · Clinical trials

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting motor neurons (MNs) in the cortex, brainstem, and spinal cord that causes weakness and atrophy of skeletal muscles [1]. While traditionally considered a purely motor disease, neuronal abnormalities in the prefrontal and temporal cortex may also lead to frontal executive dysfunction, with about 15 % of patients manifesting frontotemporal dementia [2]. The worldwide incidence of ALS is 2–4 cases per 100,000 persons, although there is some ethnic variation [3]. The disease is sporadic in about 85 % of cases and is familial in about 15 % of cases [4]. The average survival is 3–5 years from symptom onset [1]. Riluzole, the only Food and Drug Administration-approved medication for ALS, has at best modest effects [5]. Owing to the relentless nature of the disease, many therapeutics have been tested; however, most have been without success [6, 7]. Thus, interest in the potential of stem cell-based therapies has been increasing considerably in recent years.

The initial proposed use of stem cells as a therapy for ALS stemmed from the possibility of MN replacement and considered several stem cell types. All stem cells possess the capacity for self-renewal and undergo asymmetric division to give rise to a daughter cell that is capable of developing a phenotype other than that of the parent cell. Embryonic stem cells are totipotent and able to generate all cell types, whereas pluripotent stem cells give rise to a particular subset of cells [8]. Neural progenitor cells (NPCs) are pluripotent stem cells that possess an ability to achieve characteristics of neurons or glia in daughter cells [8, 9]. Given the versatility of embryonic and pluripotent stem cells, an opportunity arose to harness stem cells for the generation of new MNs for a disease like ALS with selective MN loss. Early attempts at MN replacement using NPCs and embryonic stem cells, however, were fraught with difficulty [10–12]. Although NPCs can successfully

recapitulate normal MN development, stem cell-derived MNs must survive in a potentially diseased microenvironment, integrate into descending and local circuits of motor control, grow projection axons that travel over a meter in some cases, and form functional neuromuscular junctions [10, 12]. Thus, present studies have redirected focus away from MN replacement to a "neighborhood theory", where stem cells offer a local neuroprotective role to prevent the degeneration of existing MNs.

Mechanisms by which stem cells may provide neuroprotective support include the paracrine expression of neurotrophic factors, differentiation into nondiseased supporting non-neuronal cells, including astrocytes and microglia, and differentiation into modulatory neurons that synapse on diseased MNs [13]. Sources of stem cells that continue to generate interest for therapeutic potential in ALS are embryonic stem cells, NPC lines derived from fetal or adult tissues, and non-neural progenitor cells that may moderate the MN microenvironment [14]. This has effectively translated into several human therapeutic trials, which have employed the induction of peripheral blood stem cells (PBSCs) by granulocyte colony-stimulating factor (G-CSF) treatment, autologous transplantation of mesenchymal stem cells (MSCs) derived from the bone marrow, transplantation of olfactory ensheathing cells (OECs), and, most recently, transplantation of fetal-derived human spinal cord stem cells (HSSCs) and human fetal cortex-derived NPCs modified to secrete glial-derived neurotrophic factor (GDNF).

This review will briefly touch on preclinical studies (Table 1) relevant to stem cell-based paradigms that have been successfully translated to clinical trials (Table 2). While the preclinical literature is vast regarding stem cells and their application in ALS, the relative paucity of clinical trials underscores both the challenge of our current in vitro and animal models, as well as the difficulty in conducting well-designed clinical trials for this disease. Still, many novel strategies are gaining traction and significant achievements in stem cell therapy for ALS are on the horizon.

Transitioning from Early Preclinical Studies to Current Transplantation Paradigms

The mutant Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1)-G93A transgenic mouse and rat have served as the basis for much of the preclinical work in ALS stem cell therapy. These animals are based on the first identified gene underlying familial ALS [92], and recapitulate the progressive weakness and muscle wasting associated with selective MN loss characteristic of the disease. In the earliest studies, a survival benefit was demonstrated in irradiated SOD1-G93A mice treated with human umbilical cord blood [93, 94]. Follow-up studies also showed that transplanted human cord blood along with immunosuppression with cyclosporine delayed disease progression and that the transplanted cells were detected in the brain and spinal cord [25, 26]. From these beginnings arose many strategies to harness the potential of stem cells for ALS.

Given that the goal of early stem cell therapies for ALS were directed at MN replacement, the finding that MNs derived from mouse embryonic stem cells could be grafted into a chick spinal cord and synapse with muscles was exciting [95]; however, results of similar studies in rodent models of ALS were not met with the same success, likely related to features of both ALS, as well as the challenges of reconstructing the motor system as mentioned above. For example, SOD1-G93A rats that underwent grafting of mouse embryonic stem cells into the spinal cord only exhibited a transient motor improvement that may have been due to trophic support provided by the grafted MNs to the degenerating endogenous MNs [11]. Thus, this transient improvement instead served as a springboard for studies focused on the neighborhood theory, which promotes beneficial neuronal synaptic interactions and the creation of a microenvironment that is supportive of existing MNs [10, 12]. This concept is important, especially considering the notion that ALS may not be a cell autonomous disorder, and that, in at least the mutant SOD1 form of the disease, toxicity is not limited to the MNs but also affects surrounding microglia and astrocytes, which can be manipulated with stem cell therapy [30, 96–98].

Evidence supporting the potential therapeutic benefit of altering the MN microenvironment was the focus of a number of studies that aimed to modify non-neuronal cells and offer neurotrophic factor support. Injections of hematopoietic stem cells into a mouse model that undergoes selective MN degeneration did not result in the formation of primary neural tissue, but did result in functional improvements thought to be related to a neuroprotective effect from GDNF produced by the grafted cells [22]. Further support of the microenvironment was demonstrated in chimeric mice produced by injecting wild-type embryonic stem cells into SOD1-G85R or G37R mutant mouse blastocysts. In this chimeric model, it was discovered that mutant SOD1-expressing MNs exhibited prolonged survival when surrounded by wild-type non-neuronal cells [99]. Other studies have focused on specific non-neuronal cells. In a study of astrocytes, SOD1-G37R mice with reduced mutant SOD1 expression in astrocytes exhibited delayed microglial activation that resulted in slowed disease progression [100]. Further animal models supporting the therapeutic benefit of wild-type astrocytes involved the transplantation of glial-restricted precursors into the cervical spine of SOD1-G93A mice. The result of this intervention was prolonged survival with reduced MN loss and slowed progression of motor functional declines [44]. Benefits in survival are also noted when cells modified to secrete GDNF are injected in mutant SOD1 rodent models. Interestingly, implantation of human MSCs engineered to secrete GDNF into skeletal

Table 1 Summary of preclinical stem cell studies in amyotrophic lateral sclerosis

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Lopez-Gonzales (2009) [11]	Mouse	ES	MN differentiated in vitro; GFP expressing	SOD1-G93A rat	1×10^5 cells, intraspinal	10 weeks	Ciclosporin A	Delay in motor decline by Rotarod	None	n/a	Degeneration of grafted cells at endstage
Deshpande (2006) [15]	Mouse	ES	MN differentiated in vitro; GFP expressing	Neuroadapted Sindbis virus-induced motor neuron death, rat	6×10^4 cells, intraspinal	5–7 weeks	Ciclosporin A; dibutyryl cyclic adenosine monophosphate, GDNF and/or rolipram	Recovery of grip strength in animals receiving all supplemental treatments	n/a	n/a	n/a
Harper (2004) [16]	Mouse	ES	MN differentiated in vitro; GFP expressing	Neuroadapted Sindbis virus-induced motor neuron death, rat	6×10^4 cells, intraspinal	5–7 weeks	Ciclosporin A; Rho kinase inhibitor (Y27632) or dibutyryl cyclic adenosine monophosphate	n/a	n/a	n/a	Axonal growth facilitated by dibutyryl cyclic adenosine monophosphate
Kerr (2003) [17]	Human	ES	none	Neuroadapted Sindbis virus-induced motor neuron death, rat	3×10^5 cells, intrathecal	3–4 weeks	Ciclosporin A or FK-506	Recovery of motor function by BBB and grip strength testing	n/a	Increased motor neuron survival	Possibly mediated via TGF-α or BDNF
Corti (2004) [18]	Mouse	BM	None	SOD1-G93A mouse	3×10^7 cells intraperitoneal	4 weeks	Irradiation, 800 rad	Delay in motor decline by Rotarod	10–13 days	Increased motor neurons and ventral root axons at 100 days	A large proportion of microglial cells formed
Solomon (2006) [19]	Mouse	BM	GFP expressing	SOD1-G93A mouse	5×10^6 cells, intravenous	6 weeks	Irradiation, 950–1100 rads	None	None	Large proportion of transplanted cells with microglial markers integrated into spinal cord	n/a
Ohnishi (2009) [20]	Mouse	BM	GFP expressing	SOD1-G93A mouse	6×10^7 cells intra-bone marrow	87.7–89 days	Irradiation, 6 Gy×2 doses	Delay in motor decline by grip strength meter	10–13 days	Increased motor neurons and ventral root axons in both eGFP and mutant SOD1 BM transplanted animals over wild type	Microglial markers noted in transplanted cells
Pastor (2013) [21]	Mouse	BM	GFP expressing or GDNF knockout	mdf mouse	1×10^6 cells, intramuscular	10 weeks	None	Improvement in motor function by rotarod and treadmill testing	n/a	Improvement in neuromuscular junction and muscle histology; increased motor neuron survival on the side of treated limb	Increased GDNF expression noted in spinal cord and corresponding cortex
Cabanes (2007) [22]	Mouse	BM	CD117+, GFP expressing	mdf mouse	3×10^5 cells, intraspinal	6 weeks	n/a	Improvement by footprint testing	n/a	Increase motor neuron survival	GDNF levels higher in grafted mice

Table 1 (continued)

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Corti (2010) [23]	Mouse	BM	Lin-c-kit+	SOD1-G93A mouse	1×10^6 cells intravenous	70 days	n/a	Delay in motor decline by Rotarod	16–17 days	Increased motor neuron survival (~50 %) with preserved ventral nerve root axons	Mechanism of protection possibly mediated by expression of GLT1 and elaboration of VEGF and angiopoietin 2
Pastor (2012) [24]	Mouse	BM, MSC	GFP expressing or GDNF knockout	mdf mouse	0.5–1×10^6 cells, intraspinal	n/a	None	Greater improvements seen in BM transplants over MSC transplants	n/a	Transplanted BM proliferate and retain bone marrow phenotype; MSCs underwent apoptosis	Increased GDNF expression in BM transplanted animals, functional improvements abolished in GDNF knockout BM transplant
Garbuzova-Davis (2008) [25]	Human	UCB	none	SOD1-G93A mouse	1–5×10^7 cells intravenous	7–8 weeks	Ciclosporin A	Delay and improvement in hindlimb extension and Rotarod	~13 days (2.5×10^7 cell group)	Reduced microglial density in spinal cord	n/a
Garbuzova-Davis (2003) [26]	Human	UCB	None	SOD1-G93A mouse	1×10^6 cells intravenous	9 weeks	Ciclosporin A	Delay in motor decline by extension reflex and footprint testing	None	n/a	Distributed throughout CNS and formed cells with astrocyte and neuronal markers
Souayah (2012) [27]	Human	UCB	None	SOD1-G93A mouse	1×10^8 cells, intravenous	5–6 weeks	None	n/a	n/a	n/a	Improved neuromuscular transmission by electrodiagnostic testing
Bigini (2011) [28]	Human	UCB	None	SOD1-G93A mouse; Wobbler mouse	5×10^5 cells, intraventricular	10 weeks (SOD1); 4 weeks (Wobbler)	Ciclosporin A	Slowed decline by stride length and rotarod (SOD1); slowed decline by running speed and grip strength (Wobbler)	18 days	No difference in MN (SOD1); increased MN survival (Wobbler)	n/a
Knippenberg (2012) [29]	Human	UCB	CD34+	SOD1-G93A mouse	2×10^5 cells, intraspinal	40 or 90 days	Ciclosporin A	Improvement in motor function by rotarod, stride length, and footprint analysis	6 days (12 days in females)	Increased motor neuron survival (~50 %)	No detected changes in growth factor production
Rizvanov (2011) [30]	Human	UCB	VEGF and FGF2 expressing	SOD1-G93A mouse	1×10^6 cells intravenous	24–28 weeks	n/a	n/a	n/a	n/a	VEGF-FGF2 expressing cells demonstrated astrocyte markers
Rizvanov (2008) [31]	Human	UCB	VEGF and L1CAM expressing	SOD1-G93A mouse	1×10^6 cells, intravenous	22–25 weeks	None	n/a	n/a	Transplanted cells formed endothelial cells	n/a

Table 1 (continued)

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Habisch (2007) [32]	Human	MSC, UCB	Neuroectodermal derivatives of each also used	SOD1-G93A mouse	1×10^5 cells, intrathecal	45 days	Ciclosporin A	None	None	Improved intraparenchymal incorporation with bone marrow-derived cells	n/a
Uccelli (2012) [33]	Mouse	MSC	Luciferase expressing	SOD1-G93A mouse	1×10^6 cells, intravenous	90 days	None	Improvement by rotarod, extension reflex, and motor score	17 days	Reduced ubiquitin inclusions	Decreased activated astrocyte and microglial cells, improvement in profile of oxidative stress/antioxidant enzyme expression
Forostyak (2011) [34]	Rat	MSC	GFP expressing	SOD1-G93A rat	1×10^5 cells intraspinal, 2×10^6 intravenous	16 weeks	Ciclosporin A	Slowed decline in motor function by BBB and grip strength testing	11 days	Increased motor neuron survival in treated group	n/a
Boucherie (2009) [35]	Rat	MSC	None	SOD1-G93A rat	2×10^6 cells, intrathecal	90 days	None	Delayed disease onset by motor score	16 days	Increased motor neuron survival and significant proportion formed astrocyte-like cells, with decreased microglia	Reduced expression of inflammatory mediators
Zhao (2007) [36]	Human	MSC	None	SOD1-G93A mouse	3×10^6 cells, intravenous	8 weeks	Irradiation, 6 Gy	Improved motor function by rotarod testing	18 days	Increased motor neuron survival at 16 and 20 weeks, improved CMAP amplitudes	n/a
Vercelli (2008) [37]	Human	MSC	None	SOD1-G93A mouse	1×10^5 cells, intraspinal	28 weeks	none	Improved motor function by rotarod testing	n/a	Increased motor neuron survival and decreased microglial activation	Motor improvement noted in male animals only
Kim (2010) [38]	Human	MSC	None	SOD1-G93A mouse	0.1, 2.0, and 10×10^5 cells, intrathecal	60 days	Ciclosporin A	Slowed decline by rotarod testing (10×10^5 cell group)	~6–8 days (2×10^5 and 10×10^5 cell groups)	Increased motor neuron survival	n/a
Suzuki (2008) [39]	Human	MSC	GFP, GDNF expressing	SOD1-G93A rat	3.6×10^5 cells, intramuscular split in 3 doses	80 days	Bupivacaine; ciclosporin A	Delay in motor dysfunction by BBB rating	18–28 days	Preservation of neuromuscular junctions and corresponding motor neurons	n/a
Knippenberg (2012) [40]	Human	MSC	Glucagon-like peptide 1 expressing, alginate matrix embedded	SOD1-G93A mouse	30 alginate capsules	40 days	None	Improvement in motor function by rotarod and footprint analysis	13 days	No difference in MN, reduction in reactive astrocytes and microglia	Increased expression of heat shock protein 70 in treated mice
Choi (2010) [41]	Human	MSC	Retroviral transduction of Neurogenin 1	SOD1-G93A mouse	1×10^6 cells intravenous	8, 14–16, or 13 and 15 weeks	Ciclosporin A	Delay in motor decline by rotarod	n/a	Improved motor neurons survival	n/a

Table 1 (continued)

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Morita (2008) [42]	Mouse/ Rat	OEC, MSC	GFP expressing	Leu126delTT mouse	3−4×10^5 cells, intraventricular	13–14 weeks	FK-506	None	Difference seen only in MSC-treated females	n/a	n/a
Martin (2007) [43]	Mouse	OEC	GFP expressing	SOD1-G93A mouse	0.4−120×10^5 cells, intraspinal	70 days	None	Improved motor function by running wheel activity and inclined plane test	22.3 days	Differentiated cells show markers for MNs, astrocytes, and oligodendrocytes	Axons seen to project to peripheral nerve but fail to make neuromuscular junctions
Lepore (2008) [44]	Rat	Glial restricted precursors	GLT1-overexpressing, also GLT1-null cells	SOD1-G93A rat	9×10^5 cells, intraspinal	90 days	Ciclosporin A	Delay in forelimb (site of transplantation) but not hindlimb function; slowed decline of phrenic nerve peak CMAP amplitudes	16.9 days	Increased motor neuron survival (47 % increase)	Majority astrocyte differentiation, may be mediated by GLT1 expression
Lepore (2011) [45]	Human	Glial restricted precursors	None	SOD1-G93A mouse	2−6×10^5 cells, intraspinal	50–60 days	Ciclosporin A or FK-506/ rapamycin	None	None	No difference	n/a
Corti (2007) [46]	Mouse	Primary NPC	GFP expressing; LewisX+− CXCR4+	SOD1-G93A mouse	2×10^4 cells intraspinal	70 days	n/a	Motor decline delayed, no change in slope of disease progression	22–23 days	Increased MN survival and preservation of ventral nerve roots at 110 days but not at end stage	A large proportion of neuronal cells formed, with a subset of motor neurons. Neuroprotection may be mediated by VEGF and IGF-1
Mitrecic (2010) [47]	Rat	Primary NPC	GFP expressing	SOD1-G93A rat	1×10^7 cells intravenous	14 and 26 weeks	Nitroprusside; TNF in half of treatment group	n/a	n/a	n/a	Improvement in graft survival with TNF therapy at 7 days; dominant GFAP positive cells at 7 days
Hwang (2009) [48]	Human	NPC	VEGF expressing	SOD1-G93A mouse	1×10^5 cells, intrathecal	70 days	n/a	Delay in motor decline by rotarod, paw grip endurance and extension reflex testing	12 days	n/a	Downregulation of apoptotic proteins in treated animals
Park (2009) [49]	Human	NPC	GFP with GDNF or multiple growth factors (BDNF, IGF-1, VEGF, NT-3, GDNF) expressing	SOD1-G93A mouse	Intrathecal	75 days	Ciclosporin A	none	Survival decreased in treatment group	Increased motor neuron survival with GDNF expressing cells	Sexual dimorphism noted, with female animals with worse outcomes

Table 1 (continued)

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Xu (2006) [50]	Human	NPC	None	SOD1-G93A rat	4×10^5 cells, intraspinal	62 day	FK-506	Slowed progression by BBB tests	11 days	Increased motor neuron survival (nearly 200 %)	Increased GDNF secretion in grafted animals
Xu (2009) [51]	Human	NPC	none	SOD1-G93A rat	1.6×10^5 cells, intraspinal	56 day	FK-506	n/a	n/a	n/a	n/a
Yan (2006) [52]	Human	NPC	None	SOD1-G93A mouse	8×10^5 cells, intraspinal	8 weeks	FK-506 and/or rapamycin and/or mycophenolate mofetil; or anti CD4 antibodies	Improved motor function by modified Wrathall scale with combination immunosuppressants	~3 week benefit with combination immunosuppressants	Prominently neuronal phenotype formed	Combination immunosuppression promotes survival of grafted cells, which imparts functional and survival benefit
Klein (2005) [53]	Human	NPC	GDNF expressing	SOD1-G93A rat	4.8×10^5 cells, intraspinal	90 days	Ciclosporin A	None	None	None	n/a
Suzuki (2007) [54]	Human	NPC	GDNF expressing	SOD1-G93A rat	4.8−7.2×10^5 cells, intraspinal	65 days	Ciclosporin A	none	none	Increased motor neuron survival by grafted cells; no change in neuromuscular junctions	n/a
Garbuzova-Davis (2002) [55]	Human	NPC	None	SOD1-G93A mouse	7.5×10^4 cells, intraspinal	53 days	Ciclosporin A	Suggestion of delayed motor decline by staircase testing, beam balance, open field and footprint testing	None	None	n/a
Gao (2005) [56]	Human	NPC	MN differentiated in vitro; GFP expressing	Neonatal sciatic axotomy rat	1×10^5 cells, intraspinal	2 months	Ciclosporin A	Improvement by sciatic function index	n/a	51 % transplanted cells with MN markers, and 19 % taking up retrograde marker from muscle	n/a
Hwang (2009) [48]	Human	NPC	VEGF expressing	SOD1-G93A mouse	1×10^5 cells, intrathecal	70 days	None	Delayed disease onset by rotarod, paw grip endurance and extension reflex testing	12 days	Some transplanted cells differentiated into MN-like cells	Reduced proapoptotic proteins and elevated antiapoptotic proteins
Xu (2011) [57]	Human	NPC	None	SOD1-G93A rat	2.4×10^5 cells, intraspinal	63 days	FK-506	Delayed motor decline by BBB and inclined plane testing	17 days	n/a	n/a
Hefferan (2012) [58]	Human	NPC	None	SOD1-G93A rat	1.8−2.6×10^5 cells, intraspinal	60–65 days	FK-506 and mycophenolate mofetil	Improvement in motor function by BBB scale and electrodiagnostic testing	None	Increased motor neuron survival (~43 %); decreased reactive astrocyte and microglial populations	n/a
Popescu (2013) [59]	Human	iPSC-NPC	None	SOD1-G93A rat	1×10^5 cells, intraspinal	3 months	Ciclosporin A	n/a	n/a	Cells formed neuronal phenotype, with MN-like morphology	n/a

Table 1 (continued)

Reference	Donor spec.	Cell type	Modification	Experimental model	Delivery method	Treatment age	Adjunct therapy	Functional improvement	Survival benefit	Histologic change	Other comments
Nizzardo (2014) [60]	Human	iPSC-NPC	ALDHhi-SSClo-VLA4+; GFP expressing	SOD1-G93A mouse	1×10^6 cells per dose, intravenous (multiple doses) or intrathecal (3 doses)	90 days	None	Improvement in motor function by rotarod	10 days (i.t.); 23 days (i.v.)	Increased motor neuron survival (40 %) and preservation of ventral axons (50 %)	Decreased microglial activation and astrogliosis

ALDH aldehyde dehydrogenase, *BBB* Basso-Beattie-Bresnahan scale, *BDNF* brain derived neurotrophic factor, *BM* bone marrow, *CMAP* compound muscle action potential, *ES* embryonic stem cell, *FGF* fibroblast growth factor, *GDNF* glial cell-line derived neurotrophic factor, *GFP* green fluorescent protein, *GLT1* glutamate transporter 1, *IGF-1* insulin-like growth factor 1, *iPSC* induced pluripotent stem cell, *L1CAM* L1 cell adhesion molecule, *MN* motor neuron, *MSC* mesenchymal stem cells, *NPC* neural progenitor cell, *NT-3* neurotrophin 3, *OEC* olfactory ensheathing cell, *SSC* side scatter, *TGF* transforming growth factor, *UCB* umbilical cord blood cells, *VEGF* vascular endothelial growth factor, *VLA4* very late antigen 4 (integrin alpha 4 beta 1) *SOD1* superoxide dismutase; *n/a* not applicable; *CNS* central nervous system; *i.t* intrathecal; *i.v.* intravenous

muscle were also able to support MN survival in the spinal cord [39], with synergistic effects attributed to vascular endothelial growth factor (VEGF) production [101]. Therefore, the ability of surrounding non-neuronal cells in the spinal cord and at the neuromuscular junction appears to play an important role in ALS pathogenesis, and the properties of stem cells are thereby ideally suited to achieve the goal of modulating the MN microenvironment.

Several stem cell types have since been examined for their potential efficacy in preclinical ALS models, including stem cells obtained directly from bone marrow. Direct implantation of nondiseased bone marrow into bone marrow of SOD1-G93A mice improved survival, an effect linked to the presence of non-neuronal cells derived from the transplanted tissue in the spinal cord [20]. Likewise, intraperitoneal transplantation of wild-type bone marrow into SOD1-G93A mice resulted in extensive incorporation of transplanted cells as microglia with improved animal survival [18]. Enriching the population of transplanted cells for c-kit+ stem cells allowed the infusion of cells peripherally into SOD1-G93A mice, with again improved function and survival associated with non-neuronal cells that migrated to the spinal cord [23]. Similarly, crossing SOD1-G93A mice with mice unable to generate myeloid or lymphoid cells slowed the disease course following subsequent bone marrow transplantation with wild-type SOD1-expressing cells, and this study further demonstrated that microglia in the mice receiving wild-type bone marrow transplants produced less superoxide, nitrite, and nitrate, and were therefore less neurotoxic [102]. Thus, these experiments again showed that MN pathology is not necessarily intrinsic to the MN, but may additionally rely on interactions with surrounding glial tissue.

Another potential source of stem cells are OECs isolated from the olfactory bulb, as they represent a relatively accessible source of endogenous NPCs. These cells showed promise in early models of spinal cord injury, where they promoted axonal regrowth and remyelination [103, 104]. Interestingly, transplantation of OECs isolated from the olfactory bulb of green fluorescent protein-expressing C57BL/6 mice into SOD1-G93A animals prolonged survival, and this effect was not associated with the formation of neuromuscular junctions in SOD1-G93A mice [43], suggesting again that the formation of interneurons, astrocytes, and even oligodendroglia may provide support for diseased MNs.

Studies using NPCs derived from elsewhere in the nervous system have lent further credence to a strategy of supporting existing MNs via trophic support. In one study, mouse-derived NPCs selected for those expressing the Lewis X surface marker and the chemokine receptor CXCR4 supported native MNs via VEGF and insulin-like growth factor-I (IGF-I)-mediated neuroprotective pathways [46]. Subsequent use of human NPCs modified to overexpress VEGF also favored antiapoptotic pathways over proapoptotic pathways in native

Table 2 Summary of clinical stem cell trials in amyotrophic lateral sclerosis

Stem cell type	Delivery method (target)	Dose	Patient eligibility	Patients (controls)	Planned outcomes	Results	Reference(s)
PBSC		G-CSF 2 μg/kg/day SC×5 days	Not provided	13 (0)	Primary: ALSFRS-R Secondary: CMAP amplitude (muscle(s) not specified)	Reduction in slope of decline of ALSFRS-R and CMAP amplitude postprocedure	Zhang (2009) [61]
		G-CSF 5 μg/kg/day SC×4 days repeated at months 0, 3, 6, 9	Age: 18–85 years EEC: D, Pr Symptom duration: <6 years FVC: >50 % FALS: Excluded Cognition: Normal	19 (20), but only those completing 6 months of study included in analysis Patients assessed at each month 0: 19 (20) 3: 17 (18) 6: 12 (16) 9: 9 (12)	Primary: ALSFRS-R Secondary: FVC, MMT megascore, CMAP megascore, NI, QoL; tracheostomy, death	No difference in clinical measures between treatment and placebo group No safety concerns G-CSF resulted in increased WBC count & circulating CD34+ cells	Nefussy (2010) [62]
		G-CSF 5 μg/kg/day SC every 12 h×4 days at months 0, 3, 6, 9 with 125 ml 18 % mannitol IV 4 times per day×5 days starting on day 3 of G-CSF	Age: 40–65 years EEC: D, Pr, Pr-LS Symptom duration: <12 months FVC: >80 % FALS: Excluded Cognition: FTD excluded	24 (0) Patients assessed at each month 0: 24 3: 24 6: 21 9: 20	Primary: safety and tolerability, clinical progression, and changes in chemokine and cytokine levels	No safety concerns No change in disease progression ↑ WBC & CD34+ cells in blood ↓MCP-1 in CSF/serum ↓IL-17 in CSF ↑ IP-10 in serum	Tarella (2010) [63] Chio (2011) [64]
	IV	G-CSF 300–600 μg/day SC×5–6 days Leukapheresis for target of 2.0×10^6 CD34+ cells/kg	Not clearly specified	8 (0)	Not clearly specified	No safety concerns No chance in disease progression No change in MRS NAA/Cr ratio	Cashman (2008) [65]
	Intrathecal	G-CSF administration followed by leukopheresis to isolate CD34+ cells	Case series	3 (0) Patient 1: 100 million cells via lumbar intrathecal catheter over 2 days Patient 2: 20 million cells over minutes intrathecal at L3/4 and cisterna magna Patient 3: 100 million cells intrathecal at C1/2 and lumbar region		Patient 1: 2 h loss of sensation in lower limbs, subjective speech improvements Patient 2: no change in disease Patient 3: gain in leg and neck strength	Janson (2001) [66]
	IV	Donor-mobilized CD34+ cells generated by G-CSF following total body radiation, fludarabine, and horse ATG Tacrolimus and methotrexate for GVHD prophylaxis	Age: 20–65 years EEC: D FVC: >60 % HLA-identical related donor	6 (matched historical controls)	Primary: Donor engraftment, clinical measures	Engraftment successful Cases of cutaneous and limited GVHD	Appel (2008) [67]
	Intracortical	G-CSF 300 μg/daily SC×3 days followed by isolation of CD133 cells by leukapheresis	EEC: Any FVC: Any Severe bulbar involvement excluded	10 (13) Control patients were those that did not accept treatment but met inclusion criteria, or those that applied after study completed recruitment	Primary: survival rate Secondary: ALSFRS-R	No safety concerns Improved survival in treatment group (disease duration 30.1 months in treatment group *vs* 14.3 in controls)	Martinez (2009) [68]
	Intracortical	G-CSF 300 μg/daily SC×3 days followed by isolation of CD133+ cells by leukapheresis	EEC: Any FVC: >30 %	67 (0) Appears to have included patients previously reported in Martinez 2009 [68]	Primary: Safety (not clearly specified)	2 subject deaths in postoperative period (respiratory failure, MI/SDH); otherwise procedure was well tolerated	Martinez (2012) [69]

Table 2 (continued)

Stem cell type	Delivery method (target)	Dose	Patient eligibility	Patients (controls)	Planned outcomes	Results	Reference(s)
		Bilateral injections into frontal motor cortex 3–4 cm from midline					
Bone marrow derived MSCs	Intrathecal (10 patients) Intrathecal + Intravenous (9 patients)		EEC: D Age: 25–65 years At least 5 point decline in ALSFRS in 1 year	19 (0)	Primary: Safety analysis	No safety concerns	Karussis (2010) [70]
	Intrathecal	Intrathecal administration at L2-3 or L3-4	EED: D, Pr, Pr-LS Age: >18	10 (0)	Primary: ALSFRS-R at day 90, 180, 270, 365 Secondary: ALSFRS-R subscores, time to 4-point worsening, survival	Trend towards stabilization of ALSFRS-R (individual patient characteristics are not reported such as disease duration, which could impact interpretation) No safety concerns	Prabhakar (2012) [71]
	Intramuscular, Intrathecal	MSCs induced to secrete NTFs	EEC: D, Pr, Pr-LS, Po Age: 18–75 years Disease duration <24 months ALSFRS-R>30 SVC>65 %	48 estimated (0)	Primary: Safety Secondary: change in ALSFRS-R, change in SVC	Study in process, results not yet reported	Clinical Trial
	Intraventricular	1×10^7 cells/kg	Case report of 63-year-old with definite ALS by EEC	1 (0)	Authors state ALS was too advanced to assess efficacy	No safety concerns	Baek (2012) [72]
	Intraspinal	Cells injected into thoracic cord (central part of spinal cord) 1 mm apart in 3 rows spaced by 3 mm	EEC: D Age: 21–75 years Disease duration: 6–96 months Mild-to-severe functional impairment at spinal level No or mild bulbar involvement No respiratory failure	7 (0) (+2 patients under compassionate use)	Primary: ALSFRS-R, Norris score, FVC every 3 months following 6-month lead in period	4 patients showed a reduction in the ALSFRS-R and FVC decline No safety concerns	Mazzini (2006) [73] Mazzini (2008) [74] Mazzini (2012) [75]
	Intraspinal	Same methods as 2006 study [73]	EEC: D, Pr Age: 20–65 years Disease Duration: <3 years FVC: >50 % FALS: excluded Onset: spinal	10 (0)	Primary: ALSFRS-R, MRC, respiratory assessment, MUNE, neurophysiological index, MRI, DTI, safety	No change in the rate of decline of clinical measures No safety concerns	Mazzini (2010) [76] Mazzini (2012) [75]
	Intraspinal	T3-4 injections 1–2.5 mm from midline at depth of 6 mm	EEC: D Age: 20–65 years Disease duration: 6–36 months FVC: >50 % Onset: Spinal	11 (0)	Primary: Safety Secondary: FVC, ALSRFS-R, MRC, Norris scale	No safety concerns No changes in disease progression	Blanquer (2010) [77] Blanquer (2012) [78]
	Intraspinal	C1-2 laminectomy and multiple injections at these levels	Disease duration: >6 months FALS: Excluded Rapid decline, FVC in terminal period (on mechanical ventilator or unable to speak)	13 (0)	Primary: Not specified	No safety concerns Authors report 7/13 patients improved postprocedure (no clear criteria for assessment)	Deda (2009) [79]
	Intraarterial	T-cell vaccination every 28 days for 10 doses Bone marrow harvest, following purification, one aliquot given to patient on same day "by selective	Disease duration 3–5 years	7 (0), only 5 completed full regimen	Primary: ALSFRS-R	No safety concerns Results not well reported, no apparent change in disease progression	Moviglia (2012) [80]

Table 2 (continued)

Stem cell type	Delivery method (target)	Dose	Patient eligibility	Patients (controls)	Planned outcomes	Results	Reference(s)
		intralesion infusion into the feeding artery" MSCs differentiated into NSCs and given intra-arterial					
OECs	Intracortical	OECs extracted from human fetal olfactory bulb tissue 2 million OECs injected into bilateral corona radiata	EEC: D, Pr Age: 20–70 years ALSFRS-R≥15	15 (20) Controls not randomized, rather first 15 patients served as cases and next 20 as controls; no formal matching No patients share nationality of the study (China)	Primary: ALSFRS-R at 4 months provided by patient, caregiver, and family member	Rate of decline between months 3 and 4 was slower for the treatment group compared with control Authors do not report presence/absence of adverse events; however, additional safety and efficacy outcomes reported in references [81–83] (see text)	Huang (2008) [84]
	Intracortical, Intraspinal	Intraspinal injections not standardized, reported to occur at impaired segments Authors suggest all patients received intraspinal injections, although the 2007 report suggest some patients only received intracortical injections	EEC; D, Pr Age: >18 years	507 (0) Intracortical only: 35 patients second injection; 5 patients 3 injections; 1 patient 4 injections; 1 patient 5 injections	ALSFRS-R, Norris scale, video recordings of patients, EMG, PFT	Authors report improved ALSFRS-R and respiratory measures after each treatment, although a progressive decline in ALSFRS-R continued	Chen (2007) [85] Chen (2012) [86]
NPCs	Intraspinal (phase I)	Dose escalation, see Table 3 for details Patients immunosuppressed with basiliximab mycophenolate mofetil, and tacrolimus and received tapering steroid dose following injections	Disease severity changed during trial to enroll less severely affected patients in later groups	15 (0)	Primary: Safety	No safety concerns Possible slowing of disease progression in patients without bulbar symptoms early in disease course; however, number of subjects fulfilling this criteria is small	Riley (2012) [87] Glass (2012) [88] Riley (2014) [89] Tadesse (2014) [90] Feldman (2014) [91]
	Intraspinal (phase II)	Dose escalation, see Table 3 for details Same immunosuppression regimen as phase I	EEC: D, Pr, Pr-LS Age: >18 years FVC: >60 % seated, >50 % supine SALS or FALS	15 (0)	Primary: Safety Secondary: attenuation of motor function loss, maintenance of respiratory capacity, stabilization of ALSFRS-R, reduction of spasticity/rigidity, graft survival	Study in process, results not yet reported	Clinical trial NCT01730716

ALSRFS-R ALS Functional Rating Scale Revised, *CMAP* compound muscle action potential, *Cr* creatine, *D* Definite, *EEC* El Escorial Criteria, *FALS* familial ALS, *FVC* forced vital capacity, *HLA* human leukocyte antigen, *IV* intravenous, *MMT* manual muscle strength; *MRS* magnetic resonance spectroscopy, *MSC* mesenchymal stromal cells, *NAA* N-acetylasparate, *NI* neurophysiolocal index (NI=CMAP amplitude×F-wave persistence / distal motor latency in ulnar nerves), *NPC* neural progenitor cell, *NSC* neural stem cells, *NTF* neurotrophic factors, *OECs* olfactory ensheathing cells, *Po* Possible, *Pr* Probable, *Pr-LS* Probable-Laboratory Supported, *QoL* quality of life, *SALS* sporadic ALS, *SVC* slow vital capacity; *PBSC* peripheral blood stem cell; *G-CSF* granulocyte colony stimulating factor; *SC* stem cell; *WBC* white blood cell; *FTD* frontotemporal dementia; *MCP-1* monocyte chemoattractant protein-1; *CSF* cerebrospinal fluid; *IL-17* interleukin-17; *IP-10* interferon-induced protein-10; *ATG* ATGAM; *GVHD* graft versus host disease; *MI* myocardial infarction; *SDH* subdural hematoma; *MUNE* motor unit number estimation; *MRI* magnetic resonance imaging; *DTI* diffusion tensor imaging; *MRC* Medical Research Council; *EMG* electromyographt; *PFT* pulmonary function test

MNs [48], and human NPCs modified to secret GDNF were able to confer protection when transplanted into the spinal cords of SOD1-G93A rats [53], although this did not result in continued muscle innervation [54]. Similarly, experiments with transplanted GDNF or IGF-1-secreting human NPCs rescued MNs but again did not result in improvement of motor performance or lifespan in SOD1-G93A rats [49]. Taken together, these experiments demonstrated that NPCs are able to rescue native MNs via local trophic signaling rather than replacing MNs, although more work to attain a more robust effect will be required to observe functional benefit.

Along the lines of creating a neuroprotective neighborhood, the human fetal spinal cord stem cell line NSI-566RSC has been studied for use in cellular therapy [14]. This cell line was derived from an 8-week fetal spinal cord and has the advantage of reduced teratoma formation risk compared with embryonic stem cells, as they are partially differentiated NPCs. When grafted into the SOD1-G93A rat spinal cord, these cells form both neurons and glia, synapse with native MNs, and elaborate a range of neurotrophic factors [14]. Furthermore, grafting of these cells resulted in a delay in onset and progression of disease symptoms and increased the lifespan of experimental animals [50]. Notably, the grafted cells formed gamma-aminobutyric acid-ergic neurons that synapsed with neighboring MNs in the ventral horn, although they did not form synaptic connections outside the spinal cord [51, 58]. Therefore, with the ability to form glial cells and a diversity of neuronal synaptic contacts around diseased MNs, NPCs such as NSI-566RSC have the advantage of being able to tackle the multifaceted local physiologic derangements seen in ALS.

Overall, the preclinical studies (Table 1; reviewed in [105]) underscore the potential for stem cells to modulate the microenvironment in ALS. Along with the many detailed mechanistic studies that have been performed using the various cell types described above, these preclinical data have formed the foundation for early clinical trials that seek to harness the unique attributes of stem cells to rescue diseased MNs.

Clinical Studies

Translation of stem cell therapy from the laboratory to the clinical realm requires 1) propagation of an easily accessible source of progenitor cells; 2) efficient delivery of cells to the affected areas; and 3) the ability of the cells to survive and integrate into local circuits, such that degenerating cell populations can be replaced or aberrant physiology reversed. In terms of the first requirement for suitable stem cell sources, recent studies have focused on the use of G-CSF-induced PBSCs, bone marrow-derived MSCs, OECs, and NPCs. The second point has also been addressed by a number of techniques for the collection and delivery for each stem cell population, with delivery methods including intravenous, intra-arterial, intrathecal, intracerebral, and intraspinal routes. And, finally, to the third requirement, stem cell integration must be quantifiable on a clinical scale. For ALS, this includes measurements of function such as the ALS Functional Rating Scale-Revised (ALSFRS-R), respiratory parameters, and survival, as well as the enrollment of adequate numbers to detect improvements in comparison with control treatments. To date, most studies are proof-of-concept or safety trials, and, as such, are conducted without placebo control groups. Likewise, the study sizes are small and are not powered to determine clinical efficacy. Thus, none of the reviewed studies make firm conclusions regarding improvements in the ALS disease course; however, these clinical studies represent an important first step in the development of stem cell therapies for ALS. In the following sections, the published studies involving the application of stem cells for the treatment of ALS in humans (summarized in Table 2) will be reviewed.

Granulocyte-Colony Stimulating Factor and Peripheral Blood Stem Cells

G-CSF is a hematopoietic growth factor that can mobilize CD34+ hematopoietic stem cells from the bone marrow to the peripheral blood, resulting in a population of PBSCs that can be collected for later use [63, 65, 106]. These CD34+ PBSCs were initially identified after cancer patients received hematotoxic chemotherapy [106], and it was later shown that these circulating hematopoietic progenitor cells could migrate into the central nervous system (CNS) and provide support for diseased MNs [107]. G-CSF itself is suggested to have a neuroprotective effect [108]. Thus, a number of strategies were implemented using G-CSF to mobilize or collect and redistribute PBSCs to the CNS in ALS.

Based on the presumption that PBSCs can migrate into the CNS, subcutaneous G-CSF was given to 13 patients, resulting in a slowing of disease progression evidenced by ALSFRS-R, as well as maintenance of compound muscle action potential amplitudes [61]. Another study in 17 patients comparing subcutaneous G-CSF with a placebo control arm showed elevated CD34+ PBSCs, but no difference in disease progression [62]. Similarly, the STEMALS trial utilized subcutaneous G-CSF along with a 5-day course of mannitol with the hopes of increasing permeability across the blood–brain barrier in 26 patients [63, 64]. Again, there was an increase in circulating CD34+ PBSCs, and in this trial a decrease in the proinflammatory cytokines monocyte chemoattractant protein-1 and interleukin-17, but no change in ALSFRS-R. Enriching the number of circulating PBSCs by first collecting and then readministering CD34+ cells induced by G-CSF has also been attempted. One study utilized subcutaneous G-CSF in 8 patients prior to PBSC isolation and peripheral infusion, and while no adverse effects were reported, clinical and imaging

measures did not seem to be significantly affected [65]. Finally, an aggressive strategy was attempted in 6 patients with ALS who received total body radiation followed by peripheral infusion of G-CSF-primed PBSCs from human leukocyte antigen-matched siblings [67]. Patients were immunosuppressed with methotrexate and tacrolimus, and subsequent graft-versus-host disease occurred in half of the patients. Although donor hematopoietic stem cells entered the CNS at sites of MN degeneration and engrafted as immunomodulatory cells, no clinical benefit was detected and the study was halted owing to a lack of benefit and impaired quality of life.

In contrast to the above G-CSF studies, others have examined more invasive procedures for PBSC delivery in order to bypass the blood–brain barrier. An early study tested 3 patients using a protocol of subcutaneous G-CSF therapy and isolation of CD34+ stem cells followed by intrathecal administration of the collected stem cells and showed minimal adverse effects [66]. Alternatively, a separate group focused on CD133+ cells mobilized by G-CSF followed by direct injection of the cells into cortical motor areas of the brain utilizing a frame-based or frame-less stereotactically guided needle [68, 69]. In the initial study, 10 patients were enrolled and compared with 10 control patients not accepting treatment or who applied after the study period [68]. One patient died within 10 days of surgery from a myocardial infarction. The treatment group showed an improvement in baseline ALSFRS-R scores; however, the control group had a higher ALSFRS-R score at baseline limiting a comparison. An additional 67 patients were then evaluated after having undergone the same procedure [69]. Two postoperative deaths were reported, and no outcome data were reported, but these serious adverse events suggest that further use of frontal cortex injection should be approached cautiously to minimize patient risk.

Overall, these G-CSF studies appear to demonstrate clinical safety, with a suggestion of clinical efficacy in some cases as well, and, based on preclinical models, this is an area that demonstrates therapeutic potential. It is clear, however, that these trials struggle with the technique of stem cell delivery, balancing widespread yet inefficient distribution of blood-borne PBSCs against expedient yet risky surgical methods. Hence, moving forward, this method of therapy will benefit from some agreement on G-CSF delivery strategies, as well as good clinical trial design utilizing large numbers of well-defined patient populations and standardized outcome measures.

Bone Marrow-Derived Hematopoietic Progenitor Stem Cells

Bone marrow-derived MSCs are another source of therapeutic potential in ALS [74]. MSCs may exert neuroprotective effects via paracrine, or “bystander”, mechanisms, such as the release of anti-inflammatory, anti apoptotic, and neurotrophic factors, and by influencing other cell types to take on a protective phenotype [109, 110]. MSCs further offer the advantages of 1) being an easily obtainable source; 2) possessing the ability for expansion in vitro; 3) lacking a requirement for immunosuppressive therapy to prevent rejections; and 4) having a reduced risk of malignant transformation [70]. Of note, it has also been suggested that MSCs are able to differentiate into neuron-like [111, 112] and glia-like [112, 113] lineages, although some have questioned this ability [114].

A number of studies have attempted to harness the potential of MSCs as a treatment for ALS. One study intrathecally administered MSCs obtained by bone marrow aspiration in 19 patients, and while all patients received MSCs via intrathecal lumbar puncture, 9 of the 19 patients also received intravenous MSCs [70]. No serious adverse events and a 6-month period of disease stability was reported following the procedure. In another study, 10 patients underwent isolation of bone marrow-derived MSCs, which were then administered intrathecally via lumbar puncture [71]. Again, the procedure was without serious adverse events; however, while some patients demonstrated stability of ALSFRS-R scores, others showed a decline from baseline. In addition, bone marrow-derived MSCs are the focus of an ongoing phase II clinical trial at the Mayo Clinic, Massachusetts General Hospital, and University of Massachusetts sponsored by Brainstorm-cell Therapeutics (Clinic Trial NCT02017912). In this study, patients are randomized to receive an intramuscular and intrathecal injection of autologous bone marrow-derived MSCs that are propagated ex vivo and induced to secrete neurotrophic factors. The study plans to enroll 48 patients and will evaluate safety and efficacy of the intervention.

Many other groups have also attempted more invasive methods of introducing MSCs in to the CNS. A case in which an Ommaya reservoir was utilized for intraventricular delivery of bone marrow-derived MSCs reported no adverse events [72]. Consecutive phase I studies in Italy in which bone marrow was obtained from the iliac crest and expanded in vitro prior to direct surgical implantation of the cells into the dorsal spinal cord involved 9 patients initially [73, 74], followed by an additional 10 patients [76], for a total of 19 patients [75]. In these studies, the procedure was well-tolerated and led to a slowed disease course in 6 patients; 4 of these patients were the youngest in the trials, and 2 subjects had a lower MN-predominant form of the disease. Thus, the authors suggested that slowing of the disease course in these subjects may have been a reflection of the disease phenotype and not the therapy. Another group, in Spain, evaluated 11 patients following injection of autologous bone marrow-derived MSCs into the spinal cord [77, 78]. No serious adverse events were reported nor were there any reported changes in the disease course; however, MNs near the areas of grafting showed fewer degenerative signs on histopathology. Finally,

a study in Turkey assessed patients following various routes of bone marrow-derived MSC administration and indicated that the procedure was safe, and in some cases motor improvement was also reported. [79].

Alternatively, the combination of MSCs from the bone marrow combined with T-cell vaccination has also been studied [80]. In this procedure, MSCs that were obtained from 7 patients were differentiated into NPCs and infused intra-arterially 48 h after a third T-cell vaccination dose. No serious adverse events were reported and some patients experienced a transient improvement in symptoms.

In summary, bone marrow-derived MSCs remain a viable source of cells that confer the advantage of easy expansion and manipulation in vitro for subsequent autologous transplantation. While the currently reported approaches for grafting bone marrow-derived MSCs have tended to be more invasive, these strategies circumvent some of the limitations of intravenous administration seen in the G-CSF studies. Moving forward, however, it will be important to determine therapeutic differences between G-CSF-induced PBSCs and bone marrow-derived MSCs, as well as to determine the optimum collection and delivery procedures.

OECs

OECs have been studied in China based on the preclinical evidence studying NPCs obtained from olfactory tissue [84]. In 1 study, fetal OECs were injected into the bilateral corona radiata in a nonrandomized, nonblinded cohort of patients [84]. At 4 months, reporting by the patients or caregivers indicated a reduction in disease progression rates. In a larger study involving 507 patients receiving intraspinal and intracortical injections (with many undergoing multiple intracortical injections), short- and long-term outcomes were reported [85, 86], reflecting a statistically significant increase in ALSFRS-R scores following the injections, but no improvement in pulmonary function tests. Notably, 7 Dutch patients with ALS who underwent the procedure in China were evaluated at their local institution and showed no improvement in symptoms [81]. Similarly, a patient followed in the USA who underwent the procedure had an acceleration in disease progression and also suffered from a possible brain hemorrhage and vasogenic edema at the injection site [82]. Moreover, a postmortem study examining brain tissue from 2 patients who underwent the procedure showed graft encasement and did not show evidence of axonal regeneration, neuronal differentiation, or myelination to suggest an alteration of ALS neuropathology [83].

Based on preclinical models, while the studies involving OECs may deserve further attention, these studies have not utilized good clinical study design, especially given the large number of patients enrolled. More objective measures aside from the ALSFRS-R did not show improvements and these nonblinded studies may be influenced by a number of types of bias. Thus, this therapy should continue to be evaluated with close scrutiny, with a need for further support from well-designed clinical studies.

NPCs

Based on the promising preclinical data mentioned above, a phase I, first-in-human, Food and Drug Administration-approved clinical trial utilizing NSI-566RSC has recently been completed [14, 87–89, 91]. In this study, 12 patients underwent unilateral or bilateral lumbar intraspinal transplantation surgeries and 6 patients received unilateral cervical intraspinal transplantation surgeries following a risk-escalation design. Of note, 3 patients received both lumbar and cervical transplants; therefore, the phase I study involved a total of 15 patients (Table 3). The study demonstrated safety

Table 3 Phase I and II trial design for the first-in-human, Food and Drug Administration -approved clinical trial utilizing NSI-566RSC in patients with amyotrophic lateral sclerosis

Phase I trial design					
Group	Number of patients	Subject details	Injection target	Injection details	Final cell dose
A1	3	Nonambulatory	Lumbar	5 unilateral	5×10^5
A2	3	Nonambulatory	Lumbar	10 bilateral	1×10^6
B	3	Ambulatory	Lumbar	5 unilateral	5×10^5
C/E	3	Ambulatory	Lumbar	10 bilateral	1×10^6
			Cervical	5 unilateral	5×10^5
D	3	Ambulatory	Cervical	5 unilateral	5×10^5
Phase II trial design					
Group	Number of patients	Injection target	Number of injections		Final cell dose
A	3	Cervical	10 bilateral		2×10^6
B	3	Cervical	20 bilateral		4×10^6
C	3	Cervical	20 bilateral		6×10^6
D	3	Cervical	20 bilateral		8×10^6
E	3	Lumbar and cervical	20 bilateral for each target		16×10^6

and tolerability of the surgical procedure using a customized injection platform with a floating cannula designed to reduce risk of injury to the spinal cord given cardiorespiratory motion [89, 91], and anatomical injection accuracy to the ventral horn was determined using presurgical magnetic resonance imaging evaluation (Fig. 1; [10]). As of the final outcome reporting in early 2014 [91], 6 patients died owing to disease progression, and 1 died from an unrelated congenital heart defect. Moreover, while the study was not designed to evaluate efficacy, preliminary analysis of disease monitoring in a majority of patients demonstrated slowed disease progression in multiple clinical measures, with the greatest effect on disease progression seen in those patients who received the highest number of injections/cells (Fig. 2; [91]). Briefly, ALSFRS-R measurements for the cohort of patients receiving both lumbar and cervical injections (upper panel, Fig. 2a) were converted into data points reflecting the change in ALSFRS-R per year for various 9-month windows (lower panel, Fig. 2a). For example, the presurgical window reflected disease progression rates prior to the initial lumbar surgery (green window), and windows following the transplantation surgery reflect changes in ALSFRS-R over set time frames postsurgery (see representative blue windows). Of note, the timing of the second transplantation surgery in which cells were delivered into the cervical targets are indicated by the vertical dashed lines. Taken together, this analysis reflects an improvement in the rate of decline in ALSFRS-R following both the lumbar and cervical stem cell transplants [as demonstrated by positive slope peaks (Fig. 2a)]; however, this benefit decreases over time [as noted by the trough in the plot (Fig. 2a)], suggesting that there are apparent windows of benefit, which result following cellular transplantation (Fig. 2b).

The promise of this trial has been further underscored by the recent in-depth postmortem analyses available for 6 patients [90]. DNA from transplanted cells was detected in the spinal cord of all samples near the injection sites, and nests of stem cells could also be visualized on histology (Fig. 3; [90]). Notably, the 5 patients who demonstrated a slowed progression or stabilization of disease in this phase I trial were all treated within approximately 2 years of symptom onset and had no bulbar features, suggesting that early intervention may provide a better response to this modality of stem cell treatment [91]. Again, this highlights the potential for stem cells to rescue native MNs, although the window for neuroprotection closes as the disease progresses.

Given the safety and feasibility established in the phase I trial, a phase II study began in September 2013 and ended in July 2014. The phase II trial was designed to identify the maximum tolerated dose of stem cells coupled with the

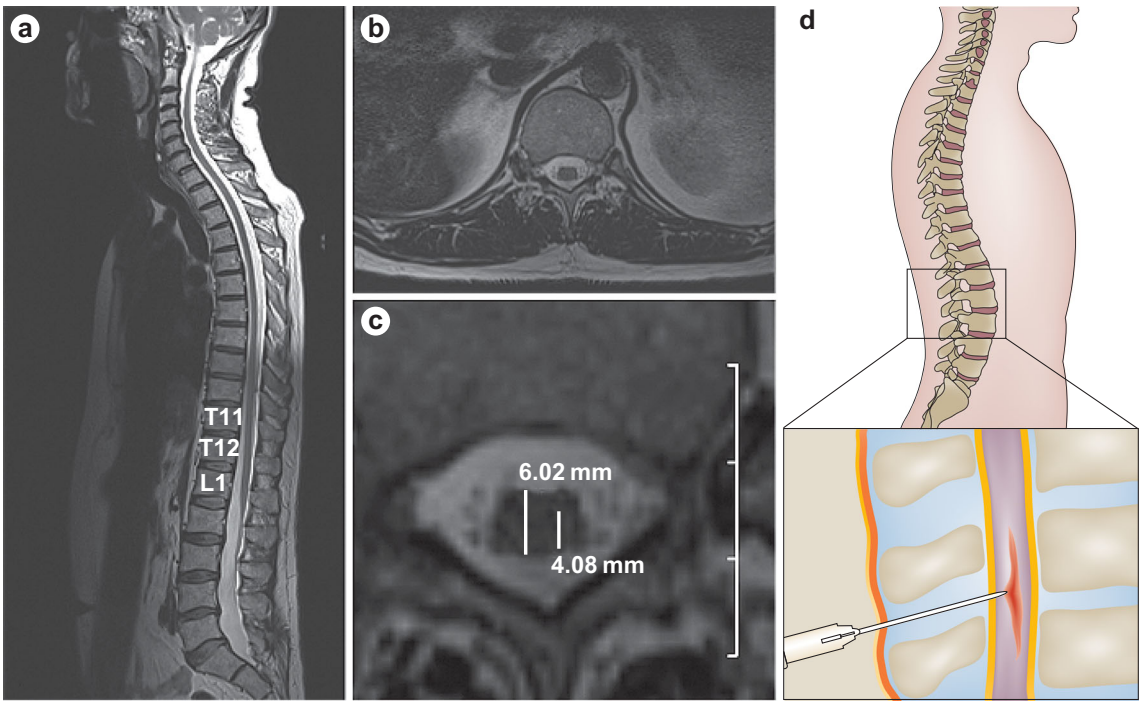
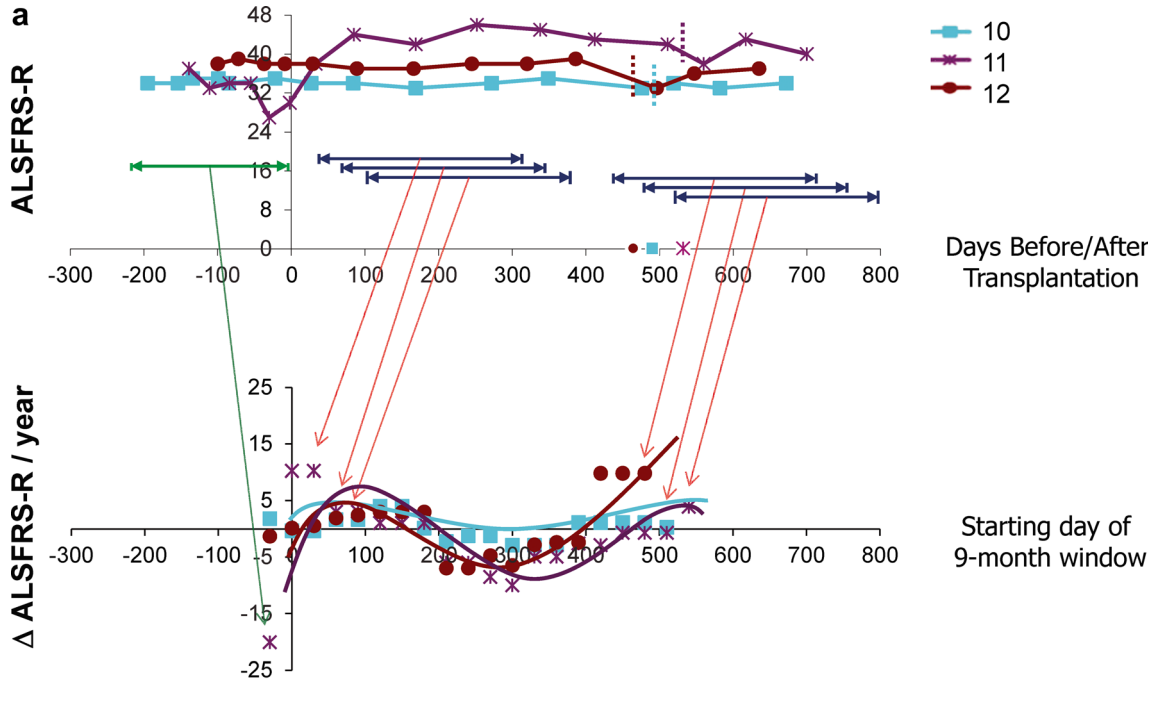


Fig. 1 Accurate anatomical targeting of stem cell delivery. **a**. T2-weighted magnetic resonance imaging scan showing a sagittal view of the spinal cord and the position of the conus medullaris and lumbar enlargement. **b**. Axial view of the spinal cord at the level of T12. **c**. Precise needle placement into the ventral horn of the spinal cord is calculated from a magnified image of part b. Estimated measurements of spinal cord diameter (6.02 mm) and distance from the dorsal root entry zone to the ventral horn (4.08 mm) are shown. Scale: 1 cm per grid division. **d**. Schematic of targeted injection of stem cells into the spinal cord. Reproduced from Boulis et al., *Nat Rev Neurol* 2011;8:172–6, [10]



b

Patient ID	Presurgery	Post-Lumbar Surgery		Post-Cervical Surgery	
	Δ ALSFRS-R (per year)	Active period	Δ ALSFRS-R (per year)	Active period	Δ ALSFRS-R (per year)
10	1.8	4-13 mos	4	0-9 mos	4.1
11	-20.1	0-9 mos	10.2	0-9 mos	3.9
12	-1.3	4-13 mos	3	0-9 mos	9.8

Fig. 2 Preliminary analysis of potential windows of human spinal stem cell (HSSC) biological activity in subjects 10–12. To identify the most biologically active period of the injected HSSCs, postsurgery data points for group E subjects were divided into a series of 9-month windows, beginning each month postsurgery, and slopes were calculated across each window. Slopes were also calculated using Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) data points for the presurgical window. **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 (i.e., window 1 corresponds to months 1–10 postsurgery, window 2 corresponds to months 2–11 postsurgery, window 3 corresponds to months 3–12 postsurgery, etc.). 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, 19.0, 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. Reproduced from Feldman et al., *Ann Neurol* 2014;75:363–73 [91]

maximum tolerated number of cervical and lumbar injections (Table 3). There were 5 treatment groups with 3 patients per group, whereby Group A received a dose of 2 million cells via 5 bilateral cervical injections (10 total), Group B progressed to 4 million cells injected in 10 bilateral cervical injections (20 total), and Groups C and D received 6 and 8 million cells, respectively, in 20 cervical injections. The final group, group E, then received 20 injections of 8 million cells into both cervical and lumbar regions, for a total of 16 million cells. All surgeries have been completed and final outcome monitoring and data review are underway; it is anticipated that an initial safety report is forthcoming, and planning is ongoing for future trials with this therapy to assess efficacy.

Induced Pluripotent Stem Cells

The ability to reprogram differentiated cells into an embryonic-like state, thus generating induced pluripotent stem (iPS) cells, was first demonstrated in 2006 [115], and has opened the door for attractive disease modeling and therapeutic strategies in ALS. Notably, the possibility of generating pluripotent iPS cells as a therapeutic option eliminates ethical concerns, as well as the risk of tissue rejection. Early preclinical studies demonstrated the successful grafting of human iPS cells into rat spinal cords, which gave rise to NPCs and, in particular, to astrocyte-like cells [116]. Given the relatively early state of this technology, however, no clinical

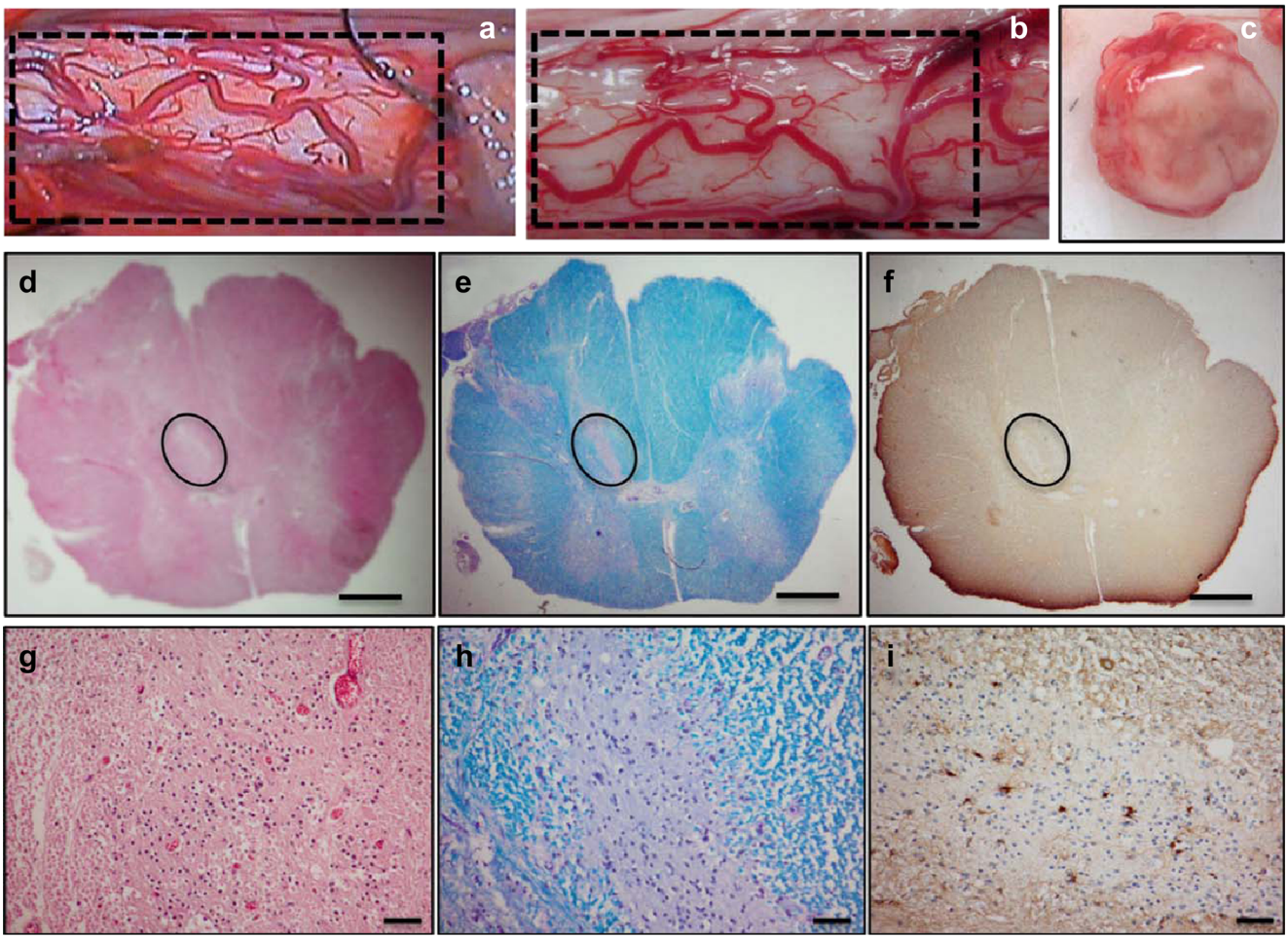


Fig. 3 Gross and histological analysis of male amyotrophic lateral sclerosis (ALS) spinal cord. Gross image of the spinal cord shows the cord surface at the site of human spinal cord stem cell (HSSC) transplant (**a**, **b**). The vascular anatomy between intraoperative videos (**a**) corresponds to the postmortem tissue (**b**). Cross-section of the cord shows no visible tissue disruption (**c**). Histological staining with hematoxylin and eosin (**d**, **g**), Luxol fast blue (**e**, **h**), and immunohistochemistry for glial fibrillary acidic protein (**f**, **i**) of 8-lm spinal cord sections from patient 4 are shown. Nest of putative HSSCs are outlined in (**d**–**f**). Scale bars: 1 mm (**d**–**f**); 50 lm (**g**–**i**). Reproduced from Tadesse et al., *Ann Clin Transl Neurol*, in press [90]

trials using iPS cells are underway, although it is likely that iPS cells will play an increasing role in ALS research in the near future. Additionally, as it has been shown that iPS cells can be generated from human dermal fibroblasts [117, 118] and many groups are capitalizing on this chance to produce embryonic-like iPS cells from patients with MN disease such as ALS [119–122]. Thus, the clinical potential of patient-derived iPS lies in the ability for patient-specific disease modeling, high-throughput drug screening, and perhaps eventually gene editing and cell replacement therapy [122, 123].

Conclusions and Future Directions

The results of preclinical studies supported the utilization of stem cell therapies as a means to modify the disease course in ALS. As previously mentioned, although early goals were set on MN replacement, preclinical models now suggest that the mechanistic benefits of stem cells in ALS favor a neighborhood theory, where secreted factors from stem cells support diseased MNs. Many results from animal models, however, have yet to be confirmed in human studies, and in order to realize the full potential of stem cells, further advancements are needed in terms of both therapeutic optimization and clinical trial design.

Moving forward, while stem cell therapeutic trials in humans are currently using a variety of cell types, state-of-the-art advances in disease modeling will advance our ability to determine which strategies represent viable treatment strategies. Notably, the ability to model ALS using iPS cells is a promising frontier because, at this time, our preclinical models mainly focus on animal models based on SOD1-G93A. Given that mutations in SOD1 only represent a very small fraction of all ALS cases, and that pathologic features may vary from familial and more frequent sporadic forms of the disease, SOD1-G93A models may not allow us to understand the full risks and benefits of potential stem cell therapies. By establishing iPS cell

lines derived directly from patients with ALS, physiologically relevant and high-throughput in vitro evaluation of treatments will allow us to further characterize ALS disease mechanisms and further understand how stem cells act as modifiers.

Next, although unrelated to stem cell therapies directly, the diagnostic delays of around 1 year typical of ALS also hinder therapeutic windows [124]. It is likely that the microenvironment is already compromised at the time symptoms develop and even more so when ALS is diagnosed; therefore, improving the time to diagnosis as a means to achieve earlier institution of therapy is essential in order to confer the best possible opportunity for MN rescue. The development of more robust biomarkers, including electrodiagnostic testing, transcranial magnetic stimulation, and advanced imaging techniques will complement advances in stem cell therapy by allowing earlier detection of disease, as well as more detailed assessments of therapeutic benefits [125, 126].

Notably, the selection of optimal outcome measures is also a challenge not unique to stem cell studies. As highlighted above and in Table 2, most studies to date, including our own, have focused on the safety and tolerability of the stem cell therapy, with efficacy remaining a secondary end point. As these trials mature and safety is established, efficacy outcomes will be expected. Assessment of MNs, graft survival, and migration of stem cells in vivo, however, is currently not directly possible, making surrogate endpoints essential. The topic of disease measurements has been the subject of a recent review [125], and is also covered elsewhere in this issue of *Neurotherapeutics*. Hence, while a full discussion of outcome measures is outside the scope of this review, a few points deserve attention. First, unlike drug trials, pharmacokinetic and pharmacodynamic markers are not available. Some groups have assessed the presence of circulating cells in the blood, although this type of evaluation is not applicable to all stem cell delivery mechanisms. In upcoming trials with cellular therapies enhanced to secrete growth factors, functional measures of these cells may be appropriate. As in many ALS trials, ALSFRS-R and survival remain viable endpoints but do not have specificity for evaluating therapy-specific efficacy measures. In other words, while ALSFRS-R can provide a good measure of patient function, it does not provide specific insight into the MN microenvironment. This highlights the importance of an autopsy to assess, in appropriate cases, graft survival and pathologic alterations in response to the therapy. The drawback is that an analysis of the native MN survival may only reflect those MNs that would have survived regardless of the intervention. Some groups have also tried to demonstrate stem cell migration via tagging procedures, and while this can show the location of these stem cells, it does not prove efficacy. As a result, we must still rely on surrogate markers. In our trial we are particularly excited about the potential insight that spinal cord MRI could provide, and certainly the modalities of diffusion tensor imaging and MR spectroscopy may be useful for other varying stem cell delivery methods as well.

Finally, good clinical trial designs will be essential to fully understand the effects and mechanisms of stem cell therapies. To date, only rare stem cell trials have advanced to a point where control groups are integrated into the study design or efficacy is being evaluated. Double-blinded, randomized, placebo-controlled studies may be possible with certain paradigms, but, with increasing invasiveness of procedures, the ethics and utility of sham procedures/surgeries becomes problematic. Still, for stem cell therapy to remain a legitimate treatment avenue, rigorous adherence to principles of clinical trial design must remain paramount. Now is a time when scientific innovation, bioengineering technologies, and medical expertise have reached a threshold, thus allowing stem cell therapy for ALS to be realized. Opportunities for new discovery remain close, and a time when stem cell therapy may turn the tide against ALS hopefully remains just over the horizon.

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