

Amyotrophic lateral sclerosis: applications of stem cells – an update

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Abstract: Neurodegenerative diseases are a growing public health challenge, and amyotrophic lateral sclerosis (ALS) remains a fatal incurable disease. The advent of stem cell therapy has opened new horizons for both researchers and ALS patients, desperately looking for a treatment. ALS must be considered a systemic disease affecting many cell phenotypes besides motor neurons, even outside the central nervous system. Cell replacement therapy needs to address the specific neurobiological issues of ALS to safely and efficiently reach clinical settings. Moreover, the enormous potential of induced pluripotent cells directly derived from patients for modeling and understanding the pathological mechanisms, in correlation with the discoveries of new genes and animal models, provides new opportunities that need to be integrated with previously described transplantation strategies. Finally, a careful evaluation of preclinical data in conjunction with wary patient choice in clinical trials needs to be established in order to generate meaningful results.

Keywords: amyotrophic lateral sclerosis, regenerative medicine, stem cell therapy, clinical trials

Introduction

Amyotrophic lateral sclerosis (ALS) still remains, more than a century since first description, a fatal and untreatable disease. The use of pluripotent or multipotent cells for the restoration of damaged neuronal networks is one of the factors that holds promise from a translational medicine perspective and a relevant part of the strongest forces driving research in stem cell (SC) biology applied to neurodegenerative diseases. Novel specific neuropathological findings in ALS suggest a more generalized involvement outside the nervous system that needs to be fully evaluated before strategies for replacement or rescue of damaged neurons can be further developed.

Amyotrophic lateral sclerosis and the frontotemporal involvement

ALS is a fatal disease caused by the progressive loss of motor neurons (MNs) in both the brain and the spinal cord leading to paralysis of voluntary muscles and death within 2–5 years from clinical onset.¹ Most cases of ALS are classified as sporadic ALS (sALS), albeit approximately 7%–10% are inherited in a dominant mode (familial ALS [fALS]).² Although several hypotheses have been proposed to explain the specific MN involvement, leading to their progressive degeneration, the underlying mechanisms remain elusive.³

ALS has been considered to be the prototypical pyramidal motor system neurodegenerative disease for decades. In terms of neuropathology, degeneration of the upper and lower motor neuron with MN cytoplasmic inclusions immunoreactive for

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ubiquitin (U) and degeneration of the corticospinal tract were considered to be diagnostic for ALS.⁴ Recently, researchers have begun to recognize an important connection between frontotemporal dementia (FTD) and ALS or Lou Gehrig's disease (Figure 1A). FTD is a syndrome of progressive changes in behavior, language, and cognition due to loss of function of neurons in both the frontal and temporal lobes. Usually, FTD has relatively little effect on the parts of the nervous system that control movement, and so many FTD patients remain physically strong and relatively agile until late in the illness. However, in approximately 10%–15% of patients with FTD, the disease also involves the nerve cells controlling voluntary movement, the MNs. When this occurs, the syndrome is called FTD with motor neuron disease (FTD-MND). TDP-43 (ubiquitinated TAR DNA-binding protein) is a multifunctional DNA/RNA-binding factor that has been implicated in the regulation of neuronal plasticity.⁵ The notion that pathological TDP-43 is involved in neurological diseases was proposed when it was discovered by Neumann et al⁶ since

this protein has been identified as the major constituent of pathological inclusions in FTD with U (FTD-U, now known as FTD-TDP), FTD-MND, and ALS. Therefore, a common pathogenesis linked to TDP-43 abnormalities in these disorders has been suggested and further confirmed.^{5,7} This scheme reflects the considerable overlap of clinicopathological features between all neurodegenerative diseases.⁸ ALS, FTD-MND, and FTD-U may be situated at different points along a continuous and broad spectrum of a multisystemic degeneration^{9,10} (Figure 1A). Recent findings of mutations in the *TARDBP* gene, encoding for TDP-43, in cases of autosomal-dominant fALS and rare sALS patients further corroborate the significance of pathological TDP-43 as being mechanistically implicated in the disease process.¹¹ Patients with both FTD-MND and *TARDBP* mutations have been reported.¹² Recently, it has been demonstrated that elevated expression of TDP-43 in mouse forebrain causes neuropathological patterns similar to FTD-U, mimicking its specific behavior phenotype.¹³ Moreover, both TDP-43 and

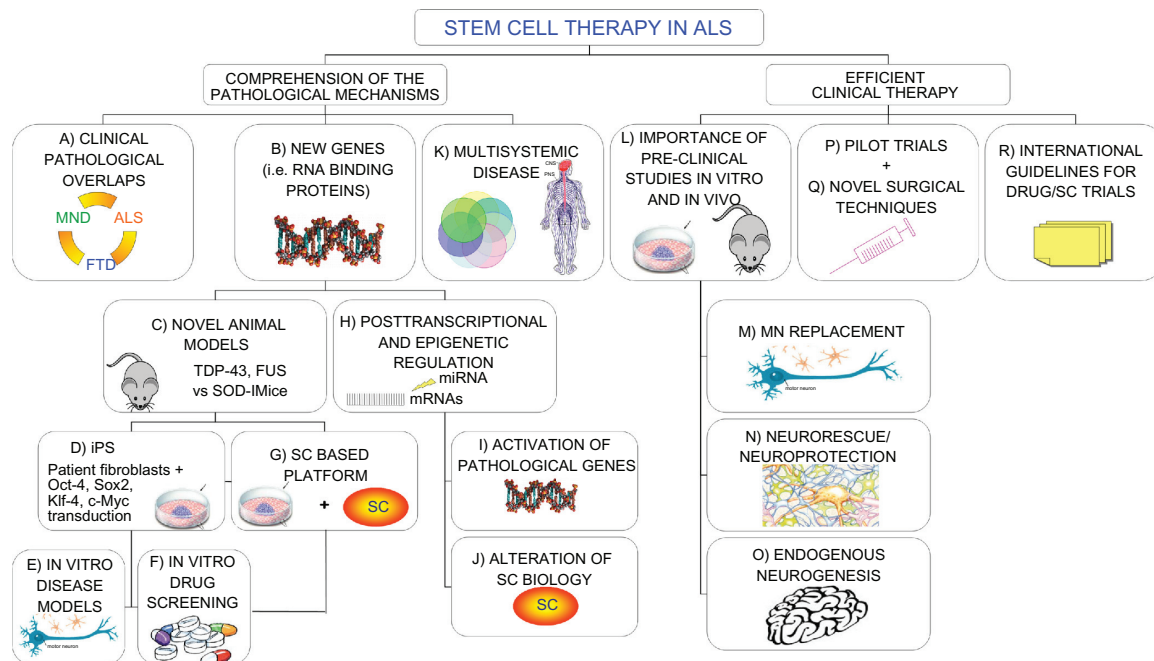


Figure 1 Novel "holistic" approach to ALS therapy. Clinical overlaps between ALS and other neurodegenerative diseases could unravel common molecular/pathological mechanisms (A). Moreover, new insights on causative genetic mutations (B) and the development of novel animal models (C) widen our knowledge of the possible therapeutic targets in the pathological pathways. In the meantime, recent iPS technology (D) provides patient-derived specimens as disease modeling and cell assays to dissect pathological mechanisms and specific cell contribution (E). The development of SC-based therapies is also directly exploitable for new drug screening (F and G). The discovery of the importance of epigenetic regulation in the pathological processes is paralleled by a relevant role in SC biology. Any alteration in this complex network could alter SC dynamic cross talk to the diseased surroundings, thus precluding possible therapeutic effects (H–J). The complex nature requires a multifaceted strategy, able to efficiently contrast widespread degeneration in all tissue districts (K), which should be carefully evaluated in accurate preclinical studies (L). Efficient therapeutic treatments are required both to replace MNs (M) and provide a healthy environment for them (N), capable also of enhancing endogenous repair (O). These laboratory studies will lead to successful clinical trials (P), based on novel surgical techniques (Q), able to slow the disease progression. Consensus international guidelines for drug/SC trials will guarantee the conscientious translation of basic SC research into appropriate treatment applications for patients aiming to create optimized efficient protocols able to slow down (neuro)degeneration (R).

Abbreviations: MND, motor neuron disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; TDP-43, TAR DNA-binding protein; FUS, fused in sarcoma protein; iPS, induced pluripotent stem cells; Oct-4/Sox2/Klf-4/c-Myc, 4 transcription factors essential for iPS generation; SC, stem cell; miRNA, micro-RNA; MN, motor neuron.

Cu/Zn superoxide dismutase-1 (SOD-1) proteins modulate sequestration of neurofilament mRNAs in the aggregates characteristic in ALS MN degeneration.¹⁴

Mutations in a gene encoding another DNA/RNA-binding protein with striking structural and functional similarities to TDP-43 named FUS (fused in sarcoma) or TLS (translocation in liposarcoma) have been recently reported to trigger degeneration of MNs^{15–17} and be responsible for FTD.¹⁸ Although this gene was initially identified as a component of a fusion pro-oncogene resulting from a chromosomal translocation seen in liposarcomas, it belongs similarly to a subfamily of RNA-binding proteins, involved in MN (patho) physiological biology/metabolism (Figure 1B). Interestingly, FUS/TLS protein interacts with RNA, single-stranded DNA, and double-stranded DNA, and is involved in unique functions in the mRNA processing and transport, transcriptional regulation, and maintenance of genomic stability.¹⁹ It has been also reported the selective presence of FUS+ inclusions in an elderly patient apparently not mutated in the gene but affected by MND.²⁰

The emerging scenario of multiple regional involvements due to the TDP-43/FUS neuropathological inclusions in the central nervous system (CNS) of ALS patients entails a significant impact on the therapeutic strategies applicable to them and, particularly, on the SC approach, since RNA-binding proteins appear as key regulators of signaling networks responsible for neuronal development and homeostasis,²¹ as well as neural SC biology.²² Therefore, healthy transplanted cells cross talk with the surroundings may also be compromised by the abnormal cellular RNA metabolism, able to trigger MN degeneration, thus impeding any therapeutic outcomes. FUS and TDP-43 harbor also a “prion domain” very similar to the specific one present in several yeast prion proteins prone to pathological misfolding transmissible within or between healthy cells or species. In this case, no SC strategy could maintain positive therapeutic outcomes in the long term, without a supportive treatment able to prevent the spread of the disease.²³ Moreover, mere cell substitution appears insufficient to contrast all the alterations in the interrelated complex pathways activated by ALS, as described in the following paragraphs.

Stem cells and new pharmacotherapeutic strategies: approaching novel genes, disease modeling, and drug candidates

Additional interesting hints derive from the discovery of the novel pathological genes *TARDBP*^{6,24} and *FUS/TLS*¹⁵ since

they also have led to the derivation of new animal models, alternatives to the classic transgenic (tg) SOD-1, for deciphering the mechanisms responsible for the motor system neurodegeneration with large implications for SC therapies in the CNS^{25,26} (Figure 1C). In particular, it appears that TDP-43 plays a pivotal role in many forms of MND, and this protein, being implicated in some forms of dementia, exerts a contributory role in a wider number of neurodegenerative diseases.²⁷ Moreover, a careful examination of the pathological SCs in these new animal models, as well as of the surrounding niche, could reveal abnormalities that may influence reparative mechanisms, as already suggested in tgSOD-1 NPs,²⁸ wobbler mice,²⁹ and in the bone marrow of sporadic ALS patients.³⁰

Furthermore, remarkable pieces of information are expected from the recent generation of iPS cells (induced pluripotent SCs, obtained by transducing cells with 4 transcription factors: Oct-4, Sox2, Klf-4, and c-Myc; Figure 1D), deriving from somatic tissues of an elderly ALS patient, which could be successfully differentiated toward well-characterized MNs.³¹ iPS cells possess the same advantages as traditional SCs due to their ability to produce differentiated affected cells, such as neurons.³² However, the use of oncogenes and retrovirus in the current iPS cell establishment protocol raises safety concerns since their progeny show high teratoma-forming propensities that actually restricts their potential use in cell therapy.³³ Nevertheless, neurodegenerative disease-specific iPS may be used for assays with cell specimens uncollectible from live patients (such as MNs, glial cells, and so on) to dissect their distinct peculiar influence on pathological events (Figure 1E) and to define new drug targets³⁴ (Figure 1F). Although reprogramming adult patient cells would be particularly interesting for both fALS and sALS, so far no ALS iPS-derived MNs appear to be affected by the disease. Conversely, both iPS cells from spinal muscular atrophy³⁵ (SMA) and familiar dysautonomia,³⁶ characterized by childhood onset, recapitulate all the pathological selective deficits. Therefore, it appears that the complex interactions between genetic and environmental features combined to senescence processes,³² which are completely lacking during iPS-derived MN differentiation, may play a key role in ALS late-onset degeneration. Additional long-term studies would be necessary to recreate the correct pathophysiological conditions before validation of this model for drug screening or as a disease model for ALS.

A precise dissection of the synergistic effects of SC implantation on the ALS surroundings is limited by the

absence of a primate model and by the still developing unique SC identification upon grafting.³⁷ Additional studies to clarify the complex stem/niche interactions could be derived from in vitro cocultures as demonstrated by the pioneering experiments of both Di Giorgio and Nagai. Human embryonic SC (ES) – derived MNs selectively die whenever cultivated in the presence of tgSOD-1 MNs or glial cells through a Bax-dependent mechanism triggered by toxic soluble factors.^{38–40} These studies may conduct to the development of standardized SC-based platforms to study cell–cell interactions and to identify novel pharmacotherapeutic targets (Figure 1G).

Innovative perspectives are derived also by the novel findings on the importance of posttranscriptional and epigenetic regulation in neurodevelopment, as well as in neurodegenerative diseases.⁴¹ Recent evidence suggests that altered RNA metabolism may play a role in ALS pathogenesis^{16,19,42} (Figure 1H). Micro-RNAs (miRNAs) are small RNA molecules highly conserved and able to regulate the expression of genes by binding to the 3'-untranslated regions of target mRNAs. miRNAs have emerged as critical regulators of gene expression, and they have been implicated in the control of virtually all the biological processes, including the biology of SCs.⁴³ Recently, it has been described how the deficit of a specific miRNA expressed in skeletal muscle (miR-206) of tgSOD-1 mice led to compromised reinnervation of neuromuscular junction with an accelerated disease progression due to an increase of denervated muscle fibers.⁴⁴ Moreover, recently a widespread modification in the total miRNA compartment after TDP-43 knockdown in culture cells has been reported, thus suggesting a direct link between the altered protein levels to specific candidate genes, crucial for TDP-43 pathogenic role in FTD and ALS.⁴⁵ Concordantly, it has been recently suggested that SC biology is also strictly controlled by some differentially expressed miRNAs that act in concerted actions to regulate self-renewal, differentiation, and division.^{46,47} As a consequence, mere cell substitution by SC progeny alone could not recover a widespread compromised transcriptional regulation responsible for disease-related gene activation. Altogether, these novel findings support the idea that a combination of approaches, related to different pathological mechanisms, is needed for effective disease modeling and, consequently, cell therapies. Conversely, SC transplantation supporting the MN surroundings in conjunction with miRNAs or RNA interference using small inhibitory RNA (siRNA) to downregulate specific pathology-involved genes (such as *TDP-43* and *GluR2*)^{48,49} or RNAs may become, therefore, an innovative

pharmacotherapeutic strategy to efficiently contrast both the MN degeneration and the environmental glial activation.

ALS viewed as a systemic disease implies new therapeutic strategies

The precise cause of ALS is still unknown, but several mutated genes have been found to predispose to ALS and recently other potential contributors to the complex pathological mechanism have been proposed.⁵⁰ Both sALS and fALS share similar clinical hallmarks, and some common genetic alterations, such as mutations in the *SOD1* or *TARDBP* genes, are also reported in sALS, thus suggesting common pathological pathways.⁵¹ Moreover, the picture arising from different studies on ALS animal models and patients, leads to the idea that multiple cell types, even outside the motor system, result as pathophysiologically affected. Beside cognitive and personality changes, indicating frontotemporal involvement, abnormalities have been described in the fibril organization compartment of the skin,^{52–54} as well as in the spinal cord collagen content of ALS patients.⁵⁵ Moreover, metalloproteinases (MMPs) involvements in different cellular districts have been reported as peculiar finding in ALS.^{56,57} Widespread variations in several MMPs and their tissue inhibitors have been demonstrated in serum and cerebrospinal fluid of ALS patients,^{58–60} around atrophic myofibers,⁶¹ in mesenchymal SCs,³⁰ and in postmortem brain/spinal cord tissues in sALS cases.⁶² Alterations of MMPs were also retrieved in tgSOD-1 mice spinal cord,⁶³ affecting both neuronal and glial cells.⁶³ Moreover, early administration of MMP synthetic inhibitors extends survival in tg mice,⁶⁴ while mutating tgSOD-1 mice knockout for a specific MMP (MMP9) show exacerbation of disease progression.⁶⁵ Since MMPs are actively responsible for all cellular signaling cascades in a variety of physiological and pathological processes,⁶⁶ their widespread variations appear to involve all cell lineages in both the sporadic and familial forms of this neurodegenerative disorder. ALS patients are also hypermetabolic and present systemic alterations of lipidemia,⁶⁷ while abnormal lipid clearance characterizes the pathological end stages of the tgSOD-1 mice.⁶⁸ Finally, it has been recently reported that mutant tgSOD-1 protein contributes to mitochondrial toxicity also in muscle tissue.⁶⁹ Interestingly, it has been recently suggested that the primary pathogenic event in tgSOD-1 mice involves muscle hypermetabolism which in turn causes neuromuscular junction destruction, followed by axonal degeneration, and finally MN loss.⁷⁰

Altogether, this evidence supports the idea of a “multisystemic” disease, affecting multiple cell types,

either neuronal or nonneuronal (Figure 1K). Notably, ALS involvement in SCs outside the motor system could represent a subject for consideration in autotransplantation of ALS patients,^{71–75} as discussed by our group in a recent article.³⁰ Major metabolic pathways (such as MMP regulation, SC biology, mitochondrial toxicity, and metabolic rate), contemporaneously affected in different cellular districts, require an appropriate therapeutic strategy able to contrast all these pathological symptoms, instead of focusing mainly on MN degeneration. Therefore, outcomes of SC transplantation may be greatly improved by a global tactic directed toward MN substitution, recovery of the surroundings and rescue effect on suffering neurons (neurorescue effect), as well as action on different pathological mechanisms outside the CNS. Today, patients suffering from ALS may have a hope to improve their quality of life by slowing down or even healing their condition through SC technology combined with pharmacological approaches. This innovative concept deeply affects the traditional approach of SC therapy based on the need for MN replacement or protection to obtain clinical recovery in ALS patients: new cellular targets appear critical to slowing down MN degeneration, as well as more widespread symptoms, due to the broad organ involvement.

MN replacement and the importance of surroundings for SC progeny in ALS

The easiest clinical strategy in treating ALS should consist of the graft of SC-derived spinal MN precursors/neuroblasts to replace damaged or dead spinal neurons. Several interesting data have been already derived from *in vitro* and *in vivo* preclinical studies (Figure 1L). MNs have been generated from different lineages of SCs including mouse and human ES *in vitro*.^{76–80} These ES-derived MNs correctly innervate muscle fibers *in vitro*,⁸¹ as well as in motor-injured adult rats.^{82,83} Motor functions have been re-established by grafted embryonic cortical neurons,⁸⁴ whereas fetal neural progenitors (NPs) may well survive and proliferate both *in vitro* and *in vivo* after transplantation.⁸⁵ Furthermore, they could be engineered to produce neurotrophins, such as glial cell-derived neurotrophic factor, to protect host MNs during the pathological progression,^{86,87} while transfection of fully differentiated MNs would be difficult and less effective. However, both ES and fetal NP cells, as well as their progeny, possess advantages and limitations that ought to be considered. Actually, ES cells display unlimited growth in culture, an undifferentiated state and great differentiation potential associated with high risk to form teratomas.

Conversely, fetal NP cells exhibit long-term stability in culture and multiple differentiation potential in conjunction with absence of tumor formation, but both ethical and immunological constraints limit their use in humans. Moreover, ALS hostile inflammatory and oxidative environment could seriously challenge fully differentiated endogenous MNs, independent of their initial origin (ES or NPs). Therefore, the harsh surroundings may seriously hamper incorporation of grafted MNS into the host neural circuitry, target axonal growth, and reinnervate pathological muscle fibers.⁸⁸

Surprisingly, both ES-derived MNs^{89,90} and NP-derived MNs^{69,91–93} transplanted in mice affected by SMA survived, integrated, and sprouted axonal terminals appropriately, thus ameliorating animal behavior, functional end points, and lifespan. These papers^{89,91} constitute the first important proof of principle that functional restoration of the motor circuit with SC-derived MNs is feasible and therapeutic through an overall neuroprotective effect associated with the decrease of proinflammatory molecules (Figure 1M). These milestone experiments also demonstrate how the complex axonal growth processes toward the skeletal muscle target could be driven and modulated by pharmacological treatment, even in adult mice. Nevertheless, many issues still need to be clarified before any clinical application: possible toxicity in human, surviving, migratory and differentiative potential of grafted cells in the degenerate environment, and their capacity to induce efficient synaptogenesis are still not fully analyzed and described.⁹⁴ Moreover, it is improbable that this approach could be soon translated in clinical trials since the restoration of the human motor circuit from MNs toward the appropriate muscle target will require considerable time (months or years) in a disease characterized by a mean survival of 36 months. Presently, it is unclear if transplanted SC-derived MNs may endure in the long term, once exposed to the harsh microenvironment of the spinal cord in ALS patients.⁸⁸ Moreover, the progressive nature of ALS, characterized by time-increased dysfunctional astrocytes and overactivated microglia, may gradually damage grafted MNs, spreading pathological hallmarks from diseased to healthy cells, as demonstrated in transplanted parkinsonian patients.⁹⁵ Actually, the importance of the surroundings during MN degeneration in ALS has been extensively proved,^{96–98} showing that disease onset can be delayed and survival increased in tgSOD-1 mice by providing genetically noncompromised supporting cells.⁹⁹ Therefore, ALS appears to be even more convincingly a noncell autonomous process wherein different cell types (such as astrocytes and microglia) play a key role in the disease progression.^{100,101} On the other hand, SCs and

their MN progeny may provide large quantities of affected MNs to define earlier pathological events relevant to disease initiation.^{102,103} As elegantly discussed in the recent review by Thonhoff et al,⁸⁸ these specimens can also be used for studying glia-mediated toxin mechanisms and test potential therapies in ALS.^{38,104} In fact, ES-derived MNs expressing mutant tgSOD-1 recapitulate the main pathological hallmarks (reduced cell survival and shortened axonal processes) and may be exploited to elucidate ALS pathophysiology or to arrest degeneration using cell-based assays^{39,105} (Figure 1G). Moreover, riddance of immature myeloid precursor cells, abnormally proliferating during late pathological phases in tgSOD-1 mice, has enhanced how this process does not primarily affect MN degeneration,¹⁰⁶ thus shifting research toward other potential targets such as inhibition of host immune or inflammatory response.¹⁰⁷ As a matter of fact, bone marrow transplant reconstituted T-cell compartment, prolonged survival, and restored glial activation within tgSOD-1 mice lacking immune modulation. These results demonstrate that the absence of immune recruitment accelerates disease progression and death, while functional CD4+ T cells provide supportive neuroprotection by modulating the trophic/cytotoxic balance of glia.¹⁰⁸ SC choice, cell dose, and delivery route (as recently reviewed)¹⁰⁷ are fundamental for a successful therapeutic strategy since subtle variations may cause divergent results.^{109,110} Nevertheless, several factors may influence enduring positive outcomes, other than structural integration in the mice motor circuitry¹¹¹ or migration to patient injury sites.¹¹² Due to its systemic and multifactorial nature, contrasting ALS entails multiple approaches able to contemporaneously act on different pathological mechanisms. As a matter of fact, effective growth release by engineered grafted SCs may be helpful to prevent MN loss, with an overall neurorescue effect able to slow down neuronal degeneration, thus avoiding pathological spreading to the surroundings. Nevertheless, additional strategies, such as SC differentiation toward glial cells, are required to preserve neuromuscular connections^{113,114} and to ameliorate pathological symptoms in ALS mice.¹¹⁵ Combining growth factor administration and SC therapy to increase healthy astroglial cell numbers also appears as a successful strategy to modulate disease progression by detoxification of the MN environment.¹¹⁴ Altogether, these new results point out the importance of multiple strategies able to counteract MN degeneration and positively influence the surrounding niche (Figure 1N).

Therefore, additional studies on the properties of SC progeny, in combination with pioneering cellular and molecular

techniques, are required to provide a trophic environment for endogenous neural cells able also to contrast the reactive astrogliosis and microglial activation.

Innovative SC therapies beyond replacement: neuroprotection by nonneural surrounding cells and activation of endogenous repair

An interesting perspective comes from the observation that NP-derived neurons support host MNs in the tgSOD-1 rat model with an advanced degree of structural integration in the motor circuit, although in the absence of any replacement or new axonal innervations into target muscle.¹¹¹ Moreover, recently it has been demonstrated that selective reduction of human SOD-1 levels in mice microglia or astrocytes, using a CRE-lox system, extends disease progression and host MN survival.^{98,99} Therefore, replacing nearby supporting cells appears a feasible strategy to preserve remaining MN functionality and activity. Transplantation of glial-restricted cell precursors by focal delivery into the ventral horn of the spinal cord extends both survival and disease duration, as well as attenuating MN loss.¹¹⁶ Interestingly, the observed neuroprotection appears partially due to normalization of the astrocyte glutamate transporter GLT1 levels, which is reduced in ALS patients and animal models.^{117,118} Concordantly, human adipose-derived SCs have the capability to increase their cytokine release when cocultured with tgSOD-1 astrocytes, which in turn show enhanced GLT1 expression and reduced caspase-3 activation, at least *in vitro*.¹¹⁹ Multiple neuroprotective effects including partial preservation of spinal cord GLT1 levels, modulation of growth factors, and microglial reduction have been recently reported after wild-type bone marrow-derived murine c-kit+ cells were systemically engrafted into tgSOD-1 mice.¹²⁰ Microglia appears as another potential target for cell transplantation in ALS since wild-type bone marrow grafted into tgSOD-1 mice, both irradiated¹²¹ or lacking CNS microglia/peripheral immune cells,¹²² augments healthy microglial content with reduction of MN loss and increased animal survival. Replacement with normal allogenic hematopoietic SCs by intra-bone marrow-bone marrow transplantation in irradiated mice also improves the neural surroundings by delaying the disease progression, in contrast to the autologous tgSOD-1 graft.¹²³ Unfortunately, hematopoietic SC transplantation in irradiated sporadic ALS patients has been demonstrated to be ineffective for improving clinical symptoms or survival, although implanted SCs correctly engraft injury sites, as demonstrated by autaptic samples.¹¹² Conversely, grafts in frontal motor cortex of

autologous multipotent hematopoietic CD-133 SCs, collected following mobilization, appear to be able to prolong ALS patient survival in a recent study,¹²⁴ although no suggested mechanisms of action are provided. Similarly, two different open-label studies using bone marrow-derived hematopoietic progenitor SCs¹²⁵ and autologous mesenchymal SCs^{72–74} report clinical benefits demonstrating that SC therapy is a safe, effective, and promising treatment for ALS patients still requiring additional evidence and standardization before being extensively applied in clinical practice.⁹⁴

Although the mammalian CNS shows a very limited capacity to regenerate after injury, endogenous precursors/SCs may provide a potential source of new neurons in the adult brain and spinal cord. As a matter of fact, endogenous precursors can differentiate into highly complex long-projection corticospinal MNs and send new projections to spinal cord targets in the healthy, adult mammalian brain, following synchronous apoptotic degeneration.¹²⁶ Alternative therapeutic strategies based on the modulation of adult neurogenesis have been already proposed and appeared promising for other neurodegenerative diseases, such as Parkinson disease (PD).⁹⁴ Few data on this specific issue have been published in ALS models, but there is convincing evidence of widespread regenerative response, mainly toward glia, in the spinal cord of tgSOD-1 mice.¹²⁷ Moreover, a temporal and regional plasticity of NPs in the dorsal horns of the spinal cord, as well as in motor cortex and lateral ventricle, in conjunction with their differentiation into neuron-like cells in response to MN loss, has been described during the disease onset or progression stages.¹²⁸ These data suggest that, when stimulated by the neurodegenerative process, the adult spinal cord possesses, at least, limited ability for regeneration, which could be potentially exploited for therapeutic purposes (Figure 10). However, this endogenous response appears impossible in contrasting widespread degeneration, but may be strategically supported by allogenic SC grafts, as recently demonstrated in a PD model where reciprocal influences between implanted cells and endogenous NPs exert multiple neurorescue effects on several brain regions.¹²⁹

Collectively, these data highlight how many potential protective strategies, alternative to MN replacement, may be explored for future applications to ALS therapy after careful testing in appropriate preclinical studies, clarifying the underlying mechanisms of action.

Caveats and the importance of patient choice in clinical SC therapy

The collected preclinical data demonstrate the feasibility of SC application to ALS animal models speeding the route

towards clinical trials, but several hurdles limit a direct translation into new therapies, as clearly discussed by Lindvall and Kokaia.⁹⁴ Retrieval of inconsistent behavioral improvements after SC grafting in animal models have not been paralleled by adequate comprehension of the underlying regulatory mechanisms exploitable for the development of standardized protocols applicable to patients. Altogether, the collected preclinical data show the feasibility of SC therapy for ALS, but more definitive answers are needed on the biological cascades activated by transplantation, such as the regulation of grafted SC behavior in terms of survival, proliferation and migration, as well as novel functional synaptogenesis, before widespread clinical trials can be contemplated.¹³⁰

Multiple small pilot trials using a variety of different SC types have been published, but inconsistency on safety procedures, optimal cell dose/source, and delivery route reduce the interpretation of their potential efficiency¹⁰⁷ (as detailed in Table 1). An impressive debate on expensive SC treatment offered worldwide has enlightened a complex reality where both meaningful interpretations and anecdotal reports are strictly linked. Nevertheless, since efficacious therapy is lacking, the severity of ALS might justify the potential risks of intervention in patients to demonstrate clinical feasibility of pioneering SC techniques.¹³¹

A clinical trial aimed to evaluate the safety of human SC implant in ALS patients is currently ongoing in the United States, which follows the preclinical validations of the surgical procedures.¹³² The SCs used in the study, prepared from cultured neural SCs, have previously been shown to extend the life of rats with ALS¹³³ and reverse the paralysis in rats affected by ischemic spastic paraplegia.¹³⁴ Direct neuronal differentiation of the grafted cells and release of growth factors to host MNs via graft–host connections have been suggested as the mechanisms responsible for the positive effects observed in both models. The phase 1 trial directed by Dr Glass at Emory University (Georgia, USA.) will enroll up to 12 ALS patients who will receive 5–10 SC injections in the lumbar area of the spinal cord. The patients will be examined at regular intervals after surgery, with final review of the data to come about 24 months later. Depending on the success of this initial trial, a follow-up phase 2 trial or a modified phase 1 trial is expected to implement the surgical and experimental procedures. The clinical trial is recruiting ALS patients (for additional data, refer Table 1).

Intravenous, intrathecal, and more often intraparenchymal administrations of hematopoietic SCs derived from peripheral blood or bone marrow have been tested in a small series of patients.^{73,112,124,125,135,136} Even if safety and lack of early side

Table 1 Recent clinical trials with SCs in ALS patients (in chronological order)

SC types	Delivery route (injection into)	Patient number	Main patient characteristics	Outcome (patient number)	Reference
Olfactory ensheathing cells	Pathological regions of the spinal cord and/or bilateral corona radiata of the brain	327	<ul style="list-style-type: none"> ➤ Onset spinal ➤ Age 20–84 ➤ Months from onset 4, 8–156 	Safe and well tolerated (short term (4 weeks)) Few side effects (16) Improvement rate of neurological function (252)	139
Olfactory ensheathing cells	Frontal lobe	1	<ul style="list-style-type: none"> ➤ Onset spinal ➤ Age 20–84 ➤ Months from onset 4, 8–156 	Faster disease rate Disabling side-effects	142
Autologous Bone marrow mesenchymal SCs	Laminectomy + mielotomy Central part of thoracic spinal cord	9	<ul style="list-style-type: none"> ➤ Onset spinal ➤ Age 32–75 ➤ Months from diagnosis 8–60 	Safe and well tolerated in the long term (4 years follow-up)	71–75
Peripheral blood SCs	Mobilization with GCSF treatment	8	<ul style="list-style-type: none"> ➤ Onset limb (7 patients) ➤ Age nr ➤ Months from onset 3–48 	Safe and well tolerated No significant changes in disease progression	135
Mobilized allogenic hematopoietic SCs	Total body irradiation Intravenous infusion Immuno-suppression	6	<ul style="list-style-type: none"> ➤ Onset spinal or bulbar ➤ Age 35–59 ➤ Months from diagnosis 5–30 	Tolerated with some immunological problems (3) No clinical benefits Immune cells in spinal cord (3 autopsies)	112
Fetal olfactory ensheathing cells	Bilateral corona radiata involving the pyramidal tracts of the frontal lobes	15	<ul style="list-style-type: none"> ➤ Onset nr ➤ Age nr ➤ Months from diagnosis/onset nr 	Safe and well tolerated (short term (4 months)) No significant difference in the rate of progression (first 2 months) Significant difference in functional deterioration (last 2 months) (7)	140
Autologous Bone marrow hematopoietic progenitors	Laminectomy Anterior part of spinal cord (C1-C2)	13	<ul style="list-style-type: none"> ➤ Onset nr ➤ Age 34–71 ➤ Months from diagnosis 8–60 	Clinical benefits (9) (1 year follow-up) No clinical benefits (1) (1 year follow-up)	125
Autologous blood purified CD133+ cells	Bilateral implantation in frontal motor cortex with stereotaxy or neuronavigation guidance	10	<ul style="list-style-type: none"> ➤ Onset spinal ➤ Age 38–62 ➤ Months from diagnosis 18–42 	Safe and well tolerated (1 year follow-up) Significant changes in disease progression vs control group	124
Fetal olfactory ensheathing cells	Bilateral corona radiata involving the pyramidal tracts of the frontal lobes	457	<ul style="list-style-type: none"> ➤ Onset nr ➤ Age nr ➤ Months from diagnosis nr 	Improvement of the patients' neurological functions and/or decrease the progressive deterioration	141
Autologous Bone marrow SCs	Laminectomy Posterior part of spinal cord	11	<ul style="list-style-type: none"> ➤ Onset medullar ➤ Age 33-61 ➤ Months from diagnosis nr 	Safe and well tolerated in the short term (some months) Transient hematoma 7 days after surgery(MRI) Grafted cells in spinal cord (2 autopsies)	143
Autologous Bone marrow SCs	Spinal tap	nr	nr	Ongoing patient enrolment	Press release: http://www.tcacellulartherapy.com/pdf/forbes_als-0110.pdf
Neural SCs	Lumbar area of spinal cord	12	Phase I trial ongoing 4 patient surgeries done (3 patients with unilateral injections, 1 patient bilateral injections into the spinal cord)	Estimated 24 months follow-up Ongoing patient enrolment Tolerated (short time)	http://www2.med.umich.edu/prmc/media/newsroom/details.cfm?ID=1306

Abbreviation: nr, not reported.

effects have been claimed, the majority of these studies did not exhibit solid preclinical evidence as recommended before translation to clinical application.^{137,138} Clinical efficacy appears unproven and long-term safety needs to be demonstrated (Figure 1P). A large number of ALS patients has been recently reported after intracerebral transplantation with olfactory ensheathing cells,^{139–141} with disabling side effects reported for a patient who received this therapy in Beijing, China.¹⁴² No sham operations have been documented, and the interpretation of the reported data is difficult because it is generated mostly outside the construct of a well-designed clinical study (see Table 1). Finally, novel surgical techniques for efficient SC delivery within the spinal cord have to be developed and tested to maximize safety and to support grafted cell integration in the host circuits, as recently suggested by several reports^{73,132,143,144} (Figure 1Q).

Guidelines for clinical trials using SC need to be specifically designed for ALS patients after selection of the most appropriate end points to reach clinical significance (Figure 1R): the historical data related to cell transplantation in PD become instrumental in achieving this goal. The neurological community has to reach a consensus on the design of clinical trials and proceed with a long follow-up to define the outcomes.

Conclusion

SC contribution in understanding the relevant pathways involved in ALS pathobiology becomes as essential as the SC application to cell therapy: their exploitation, in combination with advanced molecular techniques (ie, cell engineering, siRNA, and miRNA application), will guarantee optimized and innovative protocols for cell therapy, as well as new drug or pharmacological strategies able to influence the pathological progression of ALS. The emerging evidence of ALS as a system disease affecting, besides the CNS, also several peripheral organs in the affected patients will dictate new approaches to solve an old problem, namely the MN loss that still represents the more vulnerable side of a generalized process. Human iPS technology for generation of large numbers of MNs and other cells could help to further define generalized disease mechanism, offering powerful biological assays for drug screening. These combined efforts could broaden the chance of success, and thus, the journey that started in 1993 with the identification of the first underlying genetic defects in ALS could finally lead to successful therapy in ALS patients.

Abbreviations

ALS, amyotrophic lateral sclerosis; ES, embryonic stem cells; iPS inducible pluripotent stem cells; FTD, frontotemporal

dementia; FTD-U, frontotemporal dementia with ubiquitin inclusions; MN, motor neuron; MND, motor neuron disease; NP, neural progenitor; SC, stem cell; SMA, spinal muscular atrophy; SOD-1, superoxide dismutase; U inclusion, Ubiquitin-positive inclusion.

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Disclosure

The authors have no conflicts of interest that are directly relevant to the content of this review.

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