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Stem cells therapy for ALS

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Abstract

Introduction: Despite knowledge on the molecular basis of ALS having quickly progressed over the last few years, such discoveries have not yet translated into new therapeutics. With the advancement of stem cell technologies there is hope for stem cell therapeutics as novel treatments for ALS.

Areas Covered: we discuss in detail the therapeutic potential of different types of stem cells in pre-clinical and clinical works. Moreover we address many open questions in clinical translation.

Expert Opinion: SC therapy is a potentially promising new treatment for ALS and the need to better understand how to develop cell-based experimental treatments, and how to implement them in clinical trials, becomes more pressing. Mesenchymal Stem Cells and Neural Fetal Stem Cells have emerged as safe and potentially effective cell types but there is a need to carry out appropriately designed experimental studies to verify their long-term safety and possibly efficacy. Moreover the cost-benefit analysis of the results must take into account the quality of life of the patients as a major endpoint. It is our opinion that a multicenter international clinical program aimed at fine-tuning and coordinating transplantation procedures and protocols is mandatory.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is the most common neuromuscular disease worldwide for an incidence of 2-3 cases per 100,000 general population, and a prevalence around four to six per 100,000 [1]. It targets motor neurons (MNs) in the primary motor cortex, brainstem, and spinal cord leading to muscle atrophy, paralysis and death due to respiratory failure within 2-5 years. In most cases ALS is sporadic but a clear family history is present in approximately 10% of ALS patients. Mutations in more than 25 different genes are known to occur in 68% of familial and

about 10% of sporadic ALS [2]. ALS is a multifactorial disease and many pathogenetic mechanisms influence the onset and progression of the disease including failure of axonal transport, oxidative stress, mitochondrial dysfunction and glutamate-mediated excitotoxicity [3]. ALS was traditionally considered a pure motor disorder. However ALS rodent studies have provided strong evidence that ALS is a non-cell autonomous disease as oligodendroglia may play a significant role in onset and both astroglia and microglia play a role in progression [4]. Deletion of mutant SOD1 in astrocytes and microglia reduced disease severity and boosted the survival of ALS mice [5]. In chimeric mice expressing high levels of mutant SOD1 in 100% of MNs and oligodendrocytes, the presence of wild type support cells delayed the onset of MN degeneration [6]. Prominent neuroinflammation is found in both the central nervous system (CNS) and the spinal cords from ALS patients [7] and mouse models [8], showing gliosis and large numbers of activated microglia and astrocytes. Emerging evidence also points to an involvement of both innate and adaptive immunity in ALS progression [9-10]. Increased numbers of CD4+ and CD8+ T cells and dendritic cells were detected near dying MNs in the spinal cords and in brain parenchyma of ALS patients [11].

Our knowledge on the molecular basis of ALS has progressed very fast over the last few years, though such discoveries have not yet translated into new therapeutics. Since its approval in 1996, riluzole is indeed the only currently available drug with only modest efficacy in increasing patient survival [12]. Many clinical trials have been initiated for the ALS/MND patient community only to be abandoned later due to lack of efficacy [13].

2. Stem Cell Translational Research

As previously mentioned multiple factors are involved in the pathogenesis of ALS, but these factors have not been successfully targeted by pharmaceutical agents [13]. With the advancement of stem cell technology, stem cell therapy has been proposed as novel treatment for ALS. Stem cells (SCs) potentially target several of the putative mechanisms involved in the onset and progression of

the disease.

2.1 Cell-replacement strategies

SCs can act in neurodegenerative diseases by replacing those cells that have died, but they can also restore function through other mechanisms [14]. In the case of cell replacement, substantial improvement in ALS will require cells with the properties of motor neurons. Motor neurons can be generated *in vitro* from stem cells of various sources including embryonic stem cells (ESCs), induced pluripotent stem cells (iPS) and neural stem cells (NSCs) [15-16]. However, practical issues might limit the clinical translation of direct MNs replacement to humans. For effective cell replacement strategies for ALS these motor neurons should also reinnervate appropriate targets and establish physiologically functional synapses, send axons through inhibitory white matter, and direct axons over long distances to the target muscles in order to retain neuromuscular function. Given these limitations neuronal replacement seems unlikely to occur in ALS patients. However not only MNs but also astrocytes clearly play a role in ALS pathogenesis [5,17] and the disruption in astrocytic function markedly promotes neurodegeneration. Moreover recent studies suggest that even during normal aging, astrocytes become less supportive to motor neurons [18,19] suggesting also a role of aging in the significant motor neuron death related to astrocytes in a rodent model of familial ALS [20]. Importantly, Dass and Svendsen [20] found that priming aged wild-type and SOD1G93A astrocytes with GDNF in the media resulted in increased levels of motor neuron survival in coculture . Astrocyte precursors or stem cell-derived astrocytes promote axonal growth, support mechanisms involved in myelination and oligodendrocyte myelination , are able to modulate the host immune response, deliver neurotrophic factors and provide protective molecules against oxidative or excitotoxic insults, amongst many possible benefits [21,22,23]. Studies with chimeric mice showed that delivering wild type glial cells in the ALS model can improve the disease phenotype [6,24]. These results support the astrocyte replacement-based therapies in ALS to alleviate overall astrocyte dysfunction, deliver neurotrophic factors to degenerating spinal tissue and

stimulate endogenous CNS repair abilities [23].

2.2 Neurotrophic activity

SCs could provide a means to deliver neurotrophins to the diseased brain and spinal cord, potentially enhancing neuronal survival. One of the main mechanisms by which MNs survival is regulated in fact, consist in the release of neurotrophins by glial cells. Growth factors are proteins essential for neuronal survival: their deficiency could induce MNs death in ALS patients [25]. Among these, brain-derived neurotrophic factor (BDNF) and Glial-derived neurotrophic factor (GDNF) play critical roles in MNs survival. Interestingly, most SCs populations including mesenchymal stem cells and neural stem cells can also produce and release several neurotrophins [26,27,28,29]. Several growth factors were succesful in animal models but not in humans. Clinical trials using BDNF, ciliary neurotrophic factor, and insulin-like growth factor demonstrated no significant survival benefits [30,31,32]. These failures might be related to an inadequate route of administration. Penetration of large peptides, such as growth factors, into the CNS, in fact, is limited by the blood-brain barrier (BBB). On the other hand, SCs transplanted into the nervous system produce and deliver neurotrophic and growth factors and their efficacy could be improved by genetic modification to deliver molecules that promote MNs survival [33,34]. Spinal intrathecal transplantation of human NSCs over-expressing VEGF (F3.VEGF) in a transgenic SOD1/G93A mouse model significantly delayed disease onset and prolonged the survival of animals [35].

2.3 Anti-inflammatory activity

As previously mentioned chronic inflammation plays an important role in ALS. The most important therapeutic potential of SCs relies on their ability to regulate inflammation and to empower resident cells to facilitate tissue repair through endogenous stem cell activation or environment modulation. In fact both neural precursor cells [36] and mesenchymal stem cells

[37,38] promote “bystander” immunomodulation, as they can release soluble molecules and express immuno-relevant receptors which are able to modify the inflammatory environment. MSC transplantation was found to attenuate neuroinflammation in SOD1 G93A transgenic mice [38,39]. Many studies have demonstrated that MSCs can suppress the activation and function of various cells of the innate and adaptative immune system, including macrophages, neutrophils, natural killer cells, dendritic cells and T/B lymphocytes. These effects are mediated by several factors and molecules secreted by MSCs such as TGF-□, NO, Prostaglandins,IL- Receptor antagonists, IL-10 and many others [40,41]. The beneficial effects of glial cell replacement, the enhancement of neurotrophic support and the immunomodulatory effects suggests that SCs based therapies could prove beneficial in ALS, albeit via indirect mechanisms rather than cellular replacement [42,43].

3. Sources of Stem Cells for Clinical Trials

Candidates for stem cell therapy in ALS must be able to survive and influence the pathological tissue environment, including inflammatory and immune reactions, and migrate into the sites of diffuse neurodegeneration. Moreover, it is fundamental for clinical application that stem cells are safe, and can be easily isolated and expanded.

Mesenchymal stem cells (MSCs) are very attractive multipotent stem cells for ALS cell therapy because of their great plasticity [44] and their ability to provide the host tissue with growth factors or to modulate the host immune system [45]. They can be easily isolated from bone marrow (BM) and expanded in culture. In addition to their potential therapeutic effects, BM-derived MSCs are almost free from significant adverse effects. Most importantly, in vivo transplantation of long-term cultured hMSCs in vivo mouse models did not result in tumour formation [46]. MSC have now been tested in phase I and II clinical trials for several neurodegenerative diseases of various etiology, including ALS and safety seems to be demonstrated also in humans [47,48,49,40,51,52]. While the use of MSCs is an intriguing approach, the use of such cells from outside the CNS has been undertaken chiefly based upon the mistaken concept that a source of neural cells with stem cell characteristics may not be available, particularly for clinical applications. The source of the cells to

be transplanted represents a critical point for the implementation of cell therapy clinical trial in the CNS disorders. Clinical trials for Parkinson's disease, that used primary fetal tissue, have demonstrated, although with controversial results, that cell therapy could be suitable for neurodegenerative diseases. The use of fetal tissue presents several issues that have hampered the clinical development of this approach. In addition to the ethical concerns related to the required continuous supply of fetal specimen, the necessity to use cells from multiple fetuses in a single graft represents an additional problem. Cell viability and composition differs in donors and, further, the heterogeneity in the donor cells increases the probability of immunological rejection or contamination. An ideal tissue cell source for neural cell replacement must be renewable, thus eliminating the need for transplantation of primary fetal tissue, and must allow viability, sterility, cell composition and cell maturation to be controlled, while being inherently non tumorigenic. In order to attain a rapid clinical translation of CNS cell therapy, paramount importance must be placed upon a continuous and standardized clinical grade source of normal human neuronal cells, able to combine the plasticity of fetal tissue with an extensive proliferating capacity and functional stability. The discovery of the existence of neural stem cells (NSCs) in the adult rodent brain by Weiss and Reynolds in 1992 [53], and the initial isolation of human neural stem cells (hNSCs) by Vescovi lab in 1999 [54], have eventually provided a solution to this conundrum, thus paving the way to the implementation of perspective cell therapy applications using the brain's own stem cells. Thus, recent in vivo studies have shown that transplanted neural stem/precursor cells display good survival and integration capacity into the damaged brain parenchyma, while also eliciting putative therapeutic effects in different pathological scenarios [55, 14]. In these studies, in addition to integration and differentiation into neurons, astrocytes and oligodendrocytes, transplanted NSCs exerted their beneficial effects through an immunomodulatory action involving both innate and adaptive (local vs systemic) immune responses (eg microglial and astroglial scar reduction, T lymphocyte inhibition, etc), as well as secretion of trophic factors and cross correction of missing enzymatic activities.

Another more recent interesting source of stem cells for clinical transplantation is represented by induced Pluripotent Stem Cells (iPS). iPS can be generated from somatic cell types through ectopic expression of a defined set of transcription factors, acquiring the features of embryonic stem cells and thus bearing the potential to give rise to virtually any cell type, including inaccessible tissues such as neurons.The method was first described by Shinya Yamanaka [56] and MNs have been derived from an old patient bearing a familial form of ALS [57]. Human iPS cells might represent an ideal cell source for cell therapy given that iPS cells can be derived from the patient, thus preventing immune rejection. Therefore, iPSC technology may provide benefits in that it can allow for the use of autologous and allologous cell therapy. However iPS clinical use is still highly debated because iPS safety must be demonstrated. iPSCs have a well-known tumorigenic potential [58] moreover given the possible genetic causes of sporadic ALS a genetic alteration could be present in autologous-derived stem cells.The earliest strategies [59] for the induction of iPS cells, in fact, relied on the use of viral vectors, which however bear the risks of insertional mutagenesis and transgene reactivation, representing a limitation for clinical use. To bypass these safety concerns, numerous alternative methods for inducing pluripotency have been developed such as new small molecules recently identified that provide enhancements of somatic cell reprogramming and are able to compensate for three of the four canonical factors, SKM [60].

4. Characterization and manufacture of cell product for transplantation

To assure quality and safety of tissue and cell-based treatments, the EU Directive 2004/23/EC regarding the quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human cells and tissues was approved. This Directive was followed by Directive 2006/86/EC that regulate traceability of tissue donations and by Directive 2006/17/EC concerning the requirements for collecting of human tissues specifying tests required for donors.

Finally in 2008, European Regulation 1394/2007 came into the force as a *lex specialis,* introducing

the definition of Advanced Therapy Medicinal Products (ATMPs) that are definited as different therapies (Somatic cell therapies, Gene Therapies of Tissue engineering) that have properties for treating or preventing diseases in human beings, or that they may be used in or administered to human beings with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action.

SCs should be considered ATMPs and to be used in clinical studies they should be routinely produced according to good manufacturing practice protocols (GMP) as dictated by the European Medical Agency (EMA). This ensures that cell preparations are produced and controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release. During the whole production process, critical steps should be known and described. A thorough risk analysis during all phases of production and control ensures a final product with the expected quality. The tissue collection procedure, the cell factory and the production standard operating procedures (SOPs) and cell validation criteria must receive formal approval and certification by the appropriate regulatory body national medicinal agency in EU.

Furthermore, they require preclinical testing performed according to Good Laboratory Practice (GLP) and clinical trials conducted in Good Clinical Practice (GCP).

5. Translation into the clinic

Many promising results obtained in animal models of ALS have been lost in translation to the clinic. One problem to translate preclinical findings into new treatments for ALS patients is the lack of reproducibility of the preclinical studies. Consensus guidelines have been written in order to avoid this problem [61]. Moreover, the most common animal model of ALS, the SOD1G93A mouse, is a quite unstable model [62]. Many studies have been conducted in pre-symptomatic animals, which enhances the chances to change the disease course; nevertheless, in the human

setting no pre-symptomatic diagnostic tests are available and experimental therapies can be employed only in already symptomatic patients.

The complexity of ALS makes this motor neuron disease very difficult to treat. The lack of validated surrogate markers of disease and the great phenotypic heterogeneity delays the diagnosis. The fast progression of the neurodegenerative process in this condition leads to a very short time window for therapy administration. Also humans quite often are affected by other diseases, which add to ALS. A critical analysis of the clinical trials of proposed disease-modifying drugs in the past half-century which concluded with a large failure, there are potential methodological reasons that account for these negative results [63]. When we consider the use of stem cells for treatment, the level of complexity is further increased by the extreme physiological heterogeneity of these cells and by their unpredictable responses to the environment. Transplantation studies where human cells are implanted in animals, in fact, cannot provide full prediction of immune or other biologic responses to human cells in patients and most notably, the risk of ectopic tissue and tumor formation. Cellular transplants may persist for many years in patients, or their actions may be irreversible. Moreover pilot trials cannot be performed on normal, volunteering subjects and the use of placebo is not allowed for ethical reasons.

6. Clinical trials

Despite a great deal of positive data with stem cell transplantation in animal models, translation to human ALS patients is poor. Few phase-I/II clinical trials have been initiated based on these encouraging pre-clinical data. These trials are summarized in Table 1.

6.1 Intraparenchymal delivery

Intraparenchymal injection has been the method of choice for most clinical studies. Local injections of stem cells, close to the anterior horn of the spinal cord, have the obvious advantage of placing the cells close to their therapeutic target and favour the diffusion of trophic and

immunomodulatory factors to both the latter and the surrounding glia, thereby enhancing the likelihood of accomplishing therapeutic effects. However it is well known that ALS pathology generally starts focally and then widely spreads to the rest of the brain and spinal cord as the disease progresses [64]. This observation makes it difficult to determine the optimal target for transplantation, because multiple specific brain and spinal cord regions are affected. Transplantation in critical regions of the spinal cord involved in crucial functions such as the respiratory capacity or the control of limb movements might offer the most significant clinical benefit. Respiratory failure due to phrenic motor neuron loss is the ultimate cause of death in ALS patients [65] hence, an efficacious strategy on respiratory function could significantly modify their prognosis. A meta-analysis of 11 independent studies demonstrated that, when they were implanted somewhat close to the dying MNs, NSCs may slow both the onset and the progression of clinical signs and prolong survival in ALS mice [66] Furthermore, it was demonstrated that human neural progenitor cells (hNPC) transplanted into the ventral cervical spinal cords of SOD1G93A rats slow phrenic motor neuron cell death and increase activity in spared phrenic MN [67]. The results of the world's first clinical study to determine the safety and tolerability of direct intraparenchymal transplantation of MSCs were published in 2003 by Mazzini et al [47]. MSCs were injected with a surgical procedure into different levels of the thoracic spinal cord (T4-T5; T5-T6), of nine ALS patients. Then the study was extended to other 10 patients that were treated with the same procedures [47,48,49,50,51]. 70% of the patients manifested non-severe events (pain, tingling sensation, sensory light- touch impairment) which resolved in a few weeks. No serious adverse events were seen also in the long-term (9 years follow-up) [51] and the procedure did not accelerate disease progression. Remarkably no evidence of new masses at the injection site or anywhere else in the neuraxis was visible in any of the MRI images of the whole follow up. A similar surgical approach was performed by Blanquer [68,69] in a phase I clinical study with intraspinal injection of autologous bone marrow mononuclear cells at thoracic level (T3-T4) in 11 patients. The authors did not observe any severe transplant-related adverse events, but there were 43 non-severe events which were similar to those

reported by Mazzini et al such as temporary intercostal pain, paresthesia and dysesthesia. Twenty-two (51%) resolved in ≤ 2 weeks and only hypoestesia and constipation were still present at the end of follow-up [69]. No acceleration of the disease progression was reported. The MRI studies performed 7 days after surgery showed a transient extradural hematoma-seroma. In the follow-up studies no signs of tumour growth or post-traumatic syringomyelia were detected [68,69].

In another study, bone marrow (BM)-derived hematopoietic progenitor stem cells were injected directly into the brainstem and in the upper spinal cord of 13 ALS patients with severe bulbar involvement. In 9 patients no severe adverse events and some benefits are reported [70].

A new technique for the focal delivery of donor cells in the proximity of ventral MNs has been more recently established by means of a stabilized, stereotaxic frame. The system has been standardized in animal models using mini-pigs as a model for the human spinal cord [71]. This delivery system has been employed in the first FDA-approved trial for ALS, based on the transplantation of hNPC. In this study human spinal cord-derived neural stem cells were delivered to the spinal cord of ALS patients by direct intraparenchymal injection [72,73,74]. Twelve patients received 10 microinjections targeting the L2-L5 lumbar intraspinal injections and six patients received C3-C5 cervical-targeted intraspinal injections. Additionally, three patients underwent two surgeries receiving both lumbar and cervical HSSC transplants. This study appears to demonstrate that targeting multiple levels of the spinal cord is feasible in ALS patients [72,73,74,75] and this approach might improve therapeutic efficacy based on preclinical studies [76]. A phase II of the trial is ongoing to assess HSSC dosing and efficacy of the intervention.

The group of Vescovi in Italy [77] expanded on these studies and reported the preliminary results from a first group of six patients in a Phase I trial on ALS, in which multipotent hNSCs were isolated and reproducibly expanded from human foetal tissues obtained from spontaneous miscarriages and implanted using stereotaxic and surgical apparatus and injection procedures similar to those used by Riley and colleagues [72].The authors reported no severe side effects even if the

number of implanted cells was four and a half times higher than in previous approaches (73,74) and a floating cannula with a larger diameter was adopted [77]. This is the first report of an international coordinated effort about the cell therapy and transplantation approach in ALS patients. By utilizing a methodology similar to that previously adopted by Riley et al [74], Mazzini et al. reproduced the safety of the approach and provided an improved ability to compare the relative efficacy of the different cell types, also factoring out variance in the approach to delivery [77]. Based on these positive results, a Phase II study is planned. The results of these trials seem to demonstrate that a surgical approach to delivery of cellular therapies to the spinal cord of ALS patients can be proposed without significant adverse events. The atrophic spinal cord of ALS patients is capable of tolerating at least up to 3 ml of cell suspension in 3 injection sites [47,48,49,50]. A few side effects were reported following cell transplantation. In all trials the most common negative event is transient pain in the site of surgery hence we presume it is associated with the injection procedure itself. Other observed adverse effects were attributed to ALS progression and/or the immunosuppressive regimen.

From these studies, however, we cannot draw definitive conclusions on the safety of cells. Cell transplants may survive for several years in patients, or their effects may be irreversible. The only study currently published reporting a very long term follow-up shows the clinical and radiological results 9 years after MSCs intraspinal transplantation [51]. Long-term follow-up must consider the possibility of the development of a tumor, cyst or syrinx at the site of transplantation. Advanced MRI, in particular diffusion tensor imaging (DTI), represents an important monitoring mean because it allows a satisfactory quantification of the iatrogenic damage [78]. Although secondary to safety concerns, another major point is the monitoring of the cells after transplantation. Molecular imaging techniques, such as magnetic resonance imaging (MRI), have been explored to assess hNPC transplant location, migration and survival; however none of the techniques could be used in vivo in humans [79]. Assessment of the integrity and survival of the grafted cells, however, can be obtained from post-mortem analysis of the spinal cord. Tadesse et

al.[80] analyzed the post-mortem spinal cords of 6 patients recruited in the Neuralstem, Inc trial [73]. The presence of donor versus recipient DNA was examined using real-time PCR methods (qPCR). Fluorescence in situ hybridization (FISH) was performed using DNA probes for XY chromosomes to identify male donor HSSCs in one female case, and immunohistochemistry (IHC) was used to characterize the identified donor cells. The authors demonstrated that transplanted HSSCs survived up to 2.5 years and some cells differentiated into neurons, while others maintained their SCs phenotype [80]. Another demonstration of long-term survival of SCs after transplantation comes from another study [69]. Necroscopy was performed in three patients treated with intraspinal injection of autologous bone marrow mononuclear cells (BMNC). Pathological analysis of grafted spinal cord segments showed a greater number of MNs in the treated compared to the untreated segments. In the treated segments, MNs were surrounded by CD90+ cells and did not show degenerative ubiquitin deposits [69]. This data also provides evidence of the neurotrophic activity exerted by BMNC in the treated segments of the spinal cord. Hence autopsy would be fundamental, but consent represents an ongoing challenge, since it depends on legal and cultural aspects which vary in different countries [81].

6.2 MSCs as immunomodulatory agents: intravenous and intrathecal delivery

Although parenchymal delivery of cells has been the standard it should be emphasized that many reports support a 'touch and go' mechanism for the therapeutic effects of MSCs that does not require long-term engraftment into the CNS or other tissues [39,40]. An immediate immunomodulatory effect induced by i.v. administration of MSCs has been shown in 5 ALS patients by Karussis et al. [52]. These included an increase in CD4(+)CD25(+) regulatory cells and a reduction in the proportion of activated dendritic cells and lymphocytes and of lymphocyte proliferation. Intrathecal and combined intrathecal/intravenous transfer of autologous MSCs in patients with ALS have been tested in small phase I/II pilot studies[52,82]. Both studies concluded that this approach is safe and a slight trend toward a slowed rate of progression of the functional rating scales was observed.

6.3 Endogenous peripheral blood stem cells mobilization

Three phase I/II trials were also conducted by collecting and re-infusing granulocyte-colony stimulating factor (G-CSF) in order to mobilize peripheral blood stem cells [83,84,85] without adverse effects, but with no significant changes in disease progression. However the authors conclude that their results pave the way for a properly powered therapeutic trial with an optimized regimen of G-CSF. A phase II placebo-controlled clinical trial is ongoing in Italy.

7. Next steps for clinical translation

Future studies aimed at clinical translation of stem cell treatments should address some open questions. The number of cells to be transplanted in order to calculate a therapeutic and also a maximal tolerated ‘‘dose’’ of cells before toxicity becomes a limiting factor. One should aim to implant the largest possible number of viable therapeutic substrates (cells), so that the greatest local beneficial effect can be achieved. Also, a single dose might miss adverse events that might emerge in later trials or large therapeutic effect. Mazzini et al. [48,49,50] found no correlation between the number of transplanted cells and the incidence and severity of the side effects or the outcome. Based on the positive results of the phase I study [73,74], Neuralstem, Inc. sponsored a Phase II study which will focus on dose- escalation to define a maximum tolerated dose of NSI-566RSC cells in ALS patients.(Clinical trial identifier: NCT01730716) Fifteen patients in five different dosing cohorts will receive advancing doses of up to a maximum of 40 injections and 400,000 cells per injection. 12 patients will receive injections in the cervical spinal cord and the final three patients will receive both cervical and lumbar injections

Another controversy in SCs therapy concerns the optimal stage of the disease course for transplantation. ALS patients might benefit from transplantation before the disease has begun to spread. Nevertheless, the time window is difficult to define because of the complex mechanisms involved in the rapid progression of the disease, the heterogeneous presentation, the great

phenotypic variability and the lack of biomarkers which cause a delay in the diagnosis. Other characteristics should be carefully considered, including age and disease duration/severity at the time of the procedure. There is a tendency in experimental phase I trials to enroll patients in the advanced phases of disease, in absence of any other viable options, because they may be more motivated and have a more acceptable risk/ benefit profile than patients early in the disease course. However, the late stages of ALS are associated with significant MN damage that might create an inhospitable environment for cell therapy. Moreover patients in the late stages of disease are more susceptible to surgical complications due to disease co-morbidities. A risk-escalation paradigm in the recruitment of patients has been adopted in the Neuralstem study [73,74] and more recently in the Italian trial [77]. Under this paradigm, risk to patients receiving human spinal cord SCs transplants escalates across the different cohorts (designated A–E, with cohort A being the lowest-risk and cohort E being the highest-risk group) according to disease severity and the number and placement of injections.

Another open question is represented by the age of the patient at the time of recruitment. ALS is usually an old age related disease but considering the possible negative influence of age on the spinal cord microenvironment, the survival and trophic activity of transplanted SCs might be affected. Hence we can speculate that younger patients might benefit most from SCs transplantation.

Other open questions include the number of patients needed for efficacy trials and how to quantify a response over a short time frame. Ideally a transplanted group should be compared with one undergoing the same surgery but receiving the vehicle. Such a study is unlikely to be approved by institutional review boards in most European Countries. Although a randomized and blinded trial design is always preferable and should be undertaken whenever possible, an alternative approach is to carefully document the natural history of the disease and compare it with the outcome in transplanted patients in an open-label clinical trial. This trial design has been adopted in most pilot clinical trials conducted to date [51,69,73]

8. Expert Opinion

SC therapy is potentially a promising new treatment for ALS but the need to better understand how to develop cell-based experimental treatments, and how to implement them in clinical trials, remains more pressing. The benefit of cell therapy has been documented in many animal studies, neuronal SCs and MSCs have emerged as the most promising cell type for translational in clinical trials. However the newly developed capacity to reprogram adult iPS from patients with this disease has opened up new possibilities in this area. iPS represent a new potential sources of autologous stem cells that circumvent ethical issues and the need of immunosuppression but the clinical application of these cells needs more basic research. Their safety should be ascertained, in terms of cell proliferation, dedifferentiation, cell migration and the immune reaction they induce. Moreover they could retain a disease-specific vulnerability that will adversely affect their long-term survival and efficacy. Findings of the initial open-label studies in ALS patients are not definitive but they provide clear signals that a surgical trial may be proposed in ALS patients without significant side effects and adult stem cells both MSCs and fetal neural stem cells might be good candidates for phase II clinical trials. Unfortunately, bone marrow cells from patients with chronic diseases propagate poorly and can die prematurely. Different routes of delivery should be adopted for MSC and NSCs. The biological properties of MSCs and the results obtained in clinical trials suggest that they could have a therapeutic role in ALS as immunomodulatory agents when administered both intravenously and intrathecally. Implanted NSCs integrate, differentiate and survive predominantly as astroglial cells, which are able to release growth factors and immunomodulatory molecules and re-establish neurocircuitry. A combinatory therapeutic approach with MSC and NSCs could be proposed for future clinical trials.

Substantial challenges must be addressed and resolved to advance the use of SCs in ALS including timing of transplantation that maximizes attraction of SCs to the damaged motoneurons and determining the optimal technique for injecting SCs to enhance their survival and propagation. Moreover future efforts may focus on refining parameters of patient selection.

These studies will require close cooperation and interaction of scientists and clinicians. Optimization

of the treatment focused on the organization of large efficacy studies requires more carefully implemented exploratory trials. There is a need to carry out appropriately designed experimental studies to verify the long-term safety and possibly efficacy of these therapies. Findings of open-label studies can provide in fact clear signals of efficacy when assessment is done in an unbiased way and follow-up is extended over several years. The clinical protocol of the studies in humans using stem cells should be carefully designed so as to minimize unexpected patient-related factors that may have a negative impact on post-transplantation outcome. Moreover clinical trial designs need to be debated owing to the importance of ethical challenges and the cost-benefit analysis of the results must take into account as a major endpoint the quality of life of the patients. A stem cell therapy, in fact, will only be useful if it can manufactured at sufficient quantity and quality to treat meaningful numbers of patients and if the cost is justifiable to health care providers and insurers. It is our opinion that a multicenter clinical program aimed at fine-tuning and coordinating transplantation procedures and protocols seems mandatory to achieve more reliable and predictable outcomes post-transplantation and make cell therapy a clinical reality for ALS patients.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Article Highlights

- Stem cell therapy has been proposed as novel treatment for ALS.
- Stem cells (SCs) potentially target several of the putative mechanisms involved in the onset and progression of the disease.
- The benefit of cell therapy has been documented in many animal studies, neuronal SCs and MSCs have emerged as the most promising cell type for translational in clinical trials.
- iPS represent a new potential sources of autologous stem cells that circumvent ethical issues and the need of immunosuppression but the clinical application of these cells needs more basic research.
- Findings of the initial open-label studies in ALS patients are not definitive but they provide clear signals that a surgical trial may be proposed in ALS patients without significant side effects and adult stem cells both MSCs and fetal neural stem cells might be good candidates for phase II clinical trials.
- The author's opinion is that a multicenter clinical program aimed at fine-tuning and coordinating transplantation procedures and protocols is mandatory to achieve more reliable and predictable outcomes post-transplantation and make cell therapy a clinical reality for ALS patients.

Table 1. Clinical trials with stem cells in Amyotrophic Lateral Sclerosis

Stem cells	**Study Phase**	**Route of delivery**	**Immunosuppression treatment**	**Nb of cells**	**Nb of patients**	**Inclusion criteria**	**Outcome**	**References**
Autologous BM MSCs	I	Intraspinal T4-T9	no	Mean 57x106	19	Spinal onset, FVC>50%, ambulation with assistance or wheelchair bound Age 20-75.	Safe also in the long term (9 yrs)	Mazzini et al [47,48,49, 50,51].
Autologous BM-derived hematopoietic progenitors	I	Intraspinal T3-T4	no	2 mL mononucleated cells	11	Age:30-60 FVC>50% Spinal onset	Safe and feasible	Blanquer et al. [68,69]
OESC	II	Intracranial	Not reported	2X 10^6	15 treated 20 controls	Age: 20-70	Safe ALSFRS score stable in the first 4 months	Huang et al. [86]
Allogeneic hematopoietic stem cell (HSCT)	I/II	intravenous infusion following total body irradiation; and immuno-	Tacrolimus methotrexate		6	spinal or bulbar onset, FVC>60%, Age 35-59; Months from	Tolerated (3 chronic GVHD). No clinical benefits. Autopsies: spinal cord	Appel et al [87]

		suppression				diagnosis 5-30	engrafted with immune cells	
Autologous Bone marrow (BM)-derived hematopoietic progenitors	I/II	Intraspinal injection (C3-C4)	no	$4x10^6$ $15x10^6$ $5x10^6$	13	2-5 years from disease onset; age 34-71; "moderate or severe" symptoms, 3 pts ventilation bounded	nine pts "became much better" (improved neck and limbs MRC; EMG findings of "regeneration").	Deda et al [70]
autologous bone marrow-derived stem cells	I/II	Intrathecal	no		10	age > 18 years	Safe and feasible. Short-term follow-up of ALSFRS-R scores suggests a trend towards stabilization of disease	Prabhakar S, [82]
Autologous blood purifyed CD133(+)	I/II	bilateral implantation in frontal motor cortex,	no	$2,5\text{-}7,5x10^5$	10 and 10 controls	age 38-62; 18-42 months from diagnosis; no pts with severe bulbar involvement or malnutrition; occurrence of FVC values	safe and well-tolerated (1 year follow-up).Pts survival significantly higher than control group	Martinez et al [88]
Autologous blood purifyed CD133(+)	II	bilateral implantation in frontal motor cortex,	no	$2,5\text{-}7,5x10^5$	65	(FVC) of at least of 30% appropriate nutritional status	safe and feasible procedure.	Martinez et al [89]
Autologous BM MSCS	I/II	Intrathecally and intravenously	no	$54,7x10^6$ CSF $24,5x10^6$ iv	10 intrathecal 9 combined	Age 25-65	Feasible and safe . Immediate immunomodulatory effects.	Karussis et al [52]

Autologous BM MSCS	I	intraventricular injection	no	dose of 1 x10^5 cells/kg	1		Safe and reliable	Baek et al [90]
Mobilization of Peripheral blood stem cells (PBSC)	I/II	mobilization of autologous PBSC with GCSF	no		8	7 pts had limb onset. Time interval from onset : 3 months to 4 years. 3 pts wheelchair-bound and 5 ambulatory. Pre-treatment FVC range± 50-150%	safe and well tolerated.	Cashman et al [84]
Mobilization of Peripheral blood stem cells (PBSC)	I/II	mobilization of autologous PBSC with GCSF	no		24	Age:40-65 FVC>80% Duration<12months moderated disability	Safe and well tolerated	Chiò et al [85]
Mobilization of Peripheral blood stem cells (PBSC)	I/II	mobilization of autologous PBSC with GCSF	no		19 G-CSF 20 placebo	Age: 18-85 Duration<6years FVC>50%	Safe and well tolerated not effective in slowing down disease deterioration	Nefussy et al.[83]
Human spinal cord-derived stem cells (HSSC)	I	Intraspinal (lumbar spinal cord)	Basiliximab Prednisolone Tacrolimus Mycophenolate	5-10 injections 100,000 cells/ injection	12+6	Age >18 yrs ALSFRS-R FVC > 60%	Safe and well tolerated	Riley et al [73]Glass et al [74] Feldman et al [75]
Fetal neural stem	I	Intraspinal	Prednisolone	750,000 cells per	6	non-ambulatory	Safe and well	Mazzini et

cells		(T8-T11)	Tacrolimus	injection site.		patients FVC>60%	tolerated	al..[77]