

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Stem Cell Therapy in Motor Neuron Disease

*Alok Sharma, Hemangi Sane, Nandini Gokulchandran,
Prerna Badhe, Amruta Paranjape, Radhika Pradhan,
Rohit Das and Hema Biju*

Abstract

Motor neuron disease (MND) is an insidious, fatal disorder that progresses with the selective loss of anterior horn cells of the spinal column. Over 150 years since it was first described, various therapeutic approaches have been tested in the quest of a cure but with little success. Current standard therapy only improves lifespan by a few months; palliative care is the only option available for patients. Stem cell therapy is a potent approach for the treatment of this devastating disease. A multitude of vitalizing effects, both paracrine and somatic, a robust safety profile, as well as ease of availability make a strong case for using these cells for therapeutic purposes. Coupled with rigorous rehabilitation, this powerful treatment modality has been shown to slow disease progression, improve quality of life, and increase survival, along with being well tolerated by amyotrophic lateral sclerosis (ALS)/MND patients. Compelling preclinical as well as clinical evidence abounds that stem cells hold great potential as a therapy for ALS/MND. Although not a definitive solution yet, stem cells have been verified to have slowed and/or halted disease progression in a subset of ALS/MND patients.

Keywords: motor neuron disease (MND), amyotrophic lateral sclerosis (ALS), stem cells, stem cell therapy, neurorehabilitation, neuro-regenerative rehabilitation therapy (NRRT), bone marrow-derived stem cells (BMSCs), bone marrow-derived mononuclear cells (BMMNCs)

1. Introduction

Motor neuron disease (MND) is a set of heterogeneous, idiopathic neurodegenerative syndromes characterized by progressive degeneration of anterior horn cells of the spinal cord, clinically characterized by weak and wasting musculature, which is eventually fatal [1]. Diagnosis is confirmed via thorough neuro-electrophysiological investigations [2]. Crude incidence of ALS/MND is 1.75 (1.55–1.96)/100,000 person-years of follow-up [3]. The male/female ratio is reported to be between 1 and 3 but varies with population and age [4]. The pathophysiology is multifarious (see Section 3.1), causing poor prognosis to be the major hurdle faced by clinicians worldwide [5]. Multidisciplinary symptomatic management is the sole option that can be availed by patients [6]. Pharmacological treatment includes riluzole (glutamate inhibition) [7], edaravone (effective only in the early stages) [8], and

Nuedexta (for treating pseudobulbar affect) [9]. Multidisciplinary rehabilitation is a key in managing secondary complications of the disease [10–12].

Studies worldwide endorse the safety and efficacy of stem cells as a therapeutic intervention, for a variety of neurological disorders [13–15], including ALS/MND [16–22]. Stem cells are a potent weapon in the fight against neurodegeneration. These cells hold the unique capacity to self-renew indefinitely while also giving rise to differentiated progeny under defined physiological conditions, thus repopulating damaged tissue [23]. Exercise has also been shown to enhance the mobilization and recruitment of these cells [24]. Given these properties, harnessing the potential of stem cells as a therapy to attenuate disease progression for neurodegenerative disorders, along with customized rehabilitative regimes, has gained traction in recent years.

2. Stem cells

The defining characteristics [25] of a stem cell are the unique capabilities of the following:

2.1 Clonogenicity

Stem cells self-renew throughout life, i.e., the cells undergo symmetric division under defined physiological conditions to produce identical daughter cells and thereby maintain the stem cell pool in the organism.

2.2 Multilineage differentiation

Under certain physiological conditions, stem cells may differentiate and divide asymmetrically to yield an identical daughter cell and a nonidentical, specialized daughter cell that acquires the properties of a cell type specific to a tissue.

2.3 Tissue regeneration

Stem cells have the capacity to renew the tissues that they populate. The body contains stem cell “niches,” i.e., specific regulatory microenvironments conducive to the maintenance, proliferation, and differentiation of stem cells [26].

Depending on the source, stem cells are classified as embryonic stem cells (ESCs), fetal stem cells (FSCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). ASCs are further classified into bone marrow stem cells (BMSCs), umbilical cord stem cells (UCSCs), and adipose tissue-derived stem cells (ADSCs). ESCs are pluripotent, self-renewing cells derived from the inner mass of the preimplantation blastocyst [27]. Their most obvious benefit is their pluripotency. However, ESCs tend to be highly tumorigenic, require considerable manipulation, and are in the hotbed of ethical debates [28]. FSCs are multipotent cells obtained from fetal tissues of natural, spontaneous abortuses that undergo in utero death within a specific gestational age range [29]. Limited supply, high degree of heterogeneity in the cell viability and cellular composition, and ethical issues hamper their clinical application [30].

Among ASCs, BMSCs take the lead in stem cell therapy in a wide variety of neurological disorders owing to their robust safety profile and efficient integration into host parenchyma [31–33]. Because these are adult cells, these are easily available and are not tumorigenic. The distinctive advantage of the BMSCs over other cell types is the lack of ethical issues for acquisition and administration. Currently,

these comprise the most widely employed therapeutic strategy [19, 22, 31, 34–36]. UCSCs overcome the ethical concerns faced by the ESCs due to the ease of collection postpartum and minimal processing while posing no risk for the mother or the child. These cells, however, lose their advantage due to slow engraftment, limited single-dose availability, and long-term storage issues. Hereditary disorders further limit the benefits of UCSCs [37]. A minimally invasive subcutaneous accessibility and isolation procedure and a robust, long-term proliferation capacity outline the ADSCs' superiority [38, 39]. However, these cells find their limits in the presence of a highly heterogeneous population [40]. Pluripotent stem cells generated from cultured adult skin fibroblast cells by “inducing” dedifferentiation of unipotent, differentiated adult tissue cells by the addition of only a few defined factors are known as induced pluripotent stem cells [41]. iPSCs circumvent ethical concerns over the use of human embryos for the generation of cells of a desired tissue. However, oncogenic factors are used for induction of iPSCs' phenotype and may risk spontaneous induction of cancerous phenotypes and genomic instability [42].

3. Stem cells and motor neuron degeneration

3.1 Neuropathology of ALS/MND

Grossly, ALS/MND patients exhibit spinal cord atrophy and, in some cases, atrophy of cerebral white and gray matter (**Figure 1**). Some patients who have concomitant frontotemporal dementia show presence of cortical atrophy in frontal and temporal cortex. Microscopically, this is characterized by demyelination and axonal loss (**Figure 2**) [43–51].

3.2 Mechanism of action of stem cells

The clinical outcomes observed in ALS/MND are currently postulated to be due to various paracrine and somatic mechanisms that render a neurotrophic effect in various neurodegenerative diseases (**Figure 3**).

3.2.1 Paracrine effects

Stem cells confer neuroprotection through various paracrine mechanisms. Depending on the cellular microenvironment, these cells secrete and regulate a plethora of neurotrophic factors that are essential for the nervous system, like nerve growth factor- β (NGF- β , critical for the development and maintenance of the nervous system [52]), ciliary neurotrophic factor (CNTF, promotes neurogenesis [53]),

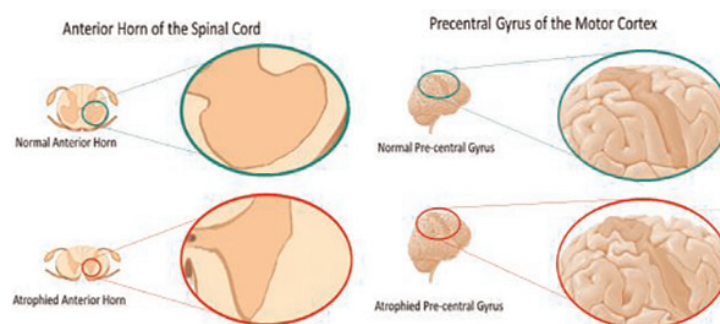


Figure 1.
The anterior horn of the spinal cord and the precentral gyrus are selectively affected and atrophy in ALS/MND.

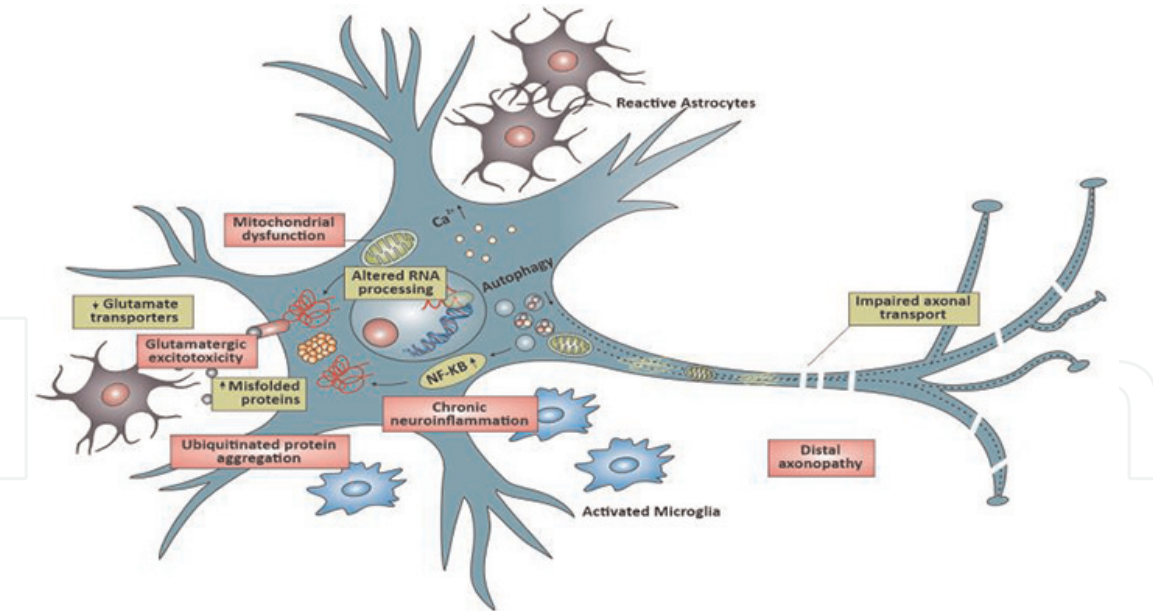


Figure 2. Pathophysiology of motor neuron disease is multifaceted. Neuronal and nonneuronal cells like glial cell dysfunction have been postulated to contribute to the pathophysiology. Oxidative stress and subsequent rise in intracellular peroxidation, upregulation of astrocytic glutamate, mitochondrial abnormality, immune dysfunction, excitotoxicity, generalized neuroinflammation due secretion of pro-inflammatory cytokines by microglia, axonal transport system dysfunction, and synaptic failure are some of the mechanisms that have been identified. Apart from these mechanisms, abnormal cytoplasmic protein inclusions in patients with ALS have highlighted genetic causality.

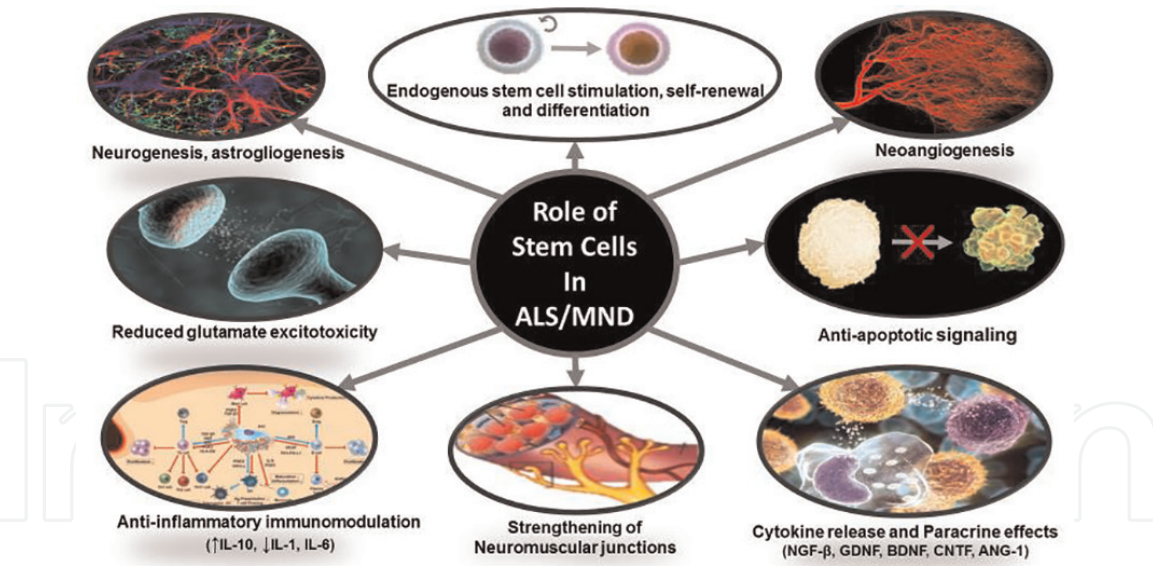


Figure 3. Stem cells play multifarious roles in mitigating ALS/MND pathology.

brain-derived neurotrophic factor (BDNF, major role player in neuronal development as well as synaptic plasticity [53]), glial cell-derived neurotrophic factor (GDNF, plays an important role in striatal dopaminergic transport [54]), and angiopoietin 1 (ANG-1, promotes angiogenesis [55]).

3.2.2 Somatic effects

They also migrate to various tissues by homing strategies and have been shown to integrate into cells of target tissue.

3.2.3 Immunomodulation

These cells exude various beneficial immunomodulatory effects and are capable of homing onto injured sites, as guided by various chemoattractant pathways [55]. Modification of the exaggerated microglial response by immunomodulatory effects is also observed. Various secreted neurotrophic factors like connective tissue growth factor (CTGF), fibroblast growth factor (FGF) 2 and 7, and various interleukins (ILs) are responsible for cell proliferation and cytoprotection. Stem cells regulate innate and adaptive immune cells through release of soluble factors such as tumor growth factor (TGF)- β and elevation of regulatory T cells (Tregs) and T-helper-2 cells (Th2 cells) [56]. Reduced levels of TNF- α , IL-1 β , IL-1 α , and IL-6 and increased levels of IL-10 lead to an anti-inflammatory effect on the neural micro-environment [56–58], enhancing neuronal repair. Soluble factors from stem cells have been shown to significantly upregulate the expression of glutamate transporters in ALS astrocytes, resulting in enhanced glutamate uptake function. Stem cells also produce vascular endothelial growth factor (VEGF), hepatic growth factor (HGF), and insulin growth factor (IGF)-1, which are reported to have neuroprotective effects [57].

3.2.4 Neurogenesis

Mezey et al. have also shown that in a strain of mice incapable of developing cells of the myeloid and lymphoid lineages, transplanted adult bone marrow cells migrated into the brain and differentiated into cells that expressed neuron-specific antigens [58].

3.2.5 Oligodendrogenesis

Using cell fate tracking techniques, Sasaki and colleagues show that stem cells can differentiate into an oligodendroglial myelinating phenotype in vivo and repair demyelinated CNS [59].

3.2.6 Astrogliogenesis

Eglitis and Mezey have demonstrated the ability of hematopoietic stem cells (HSCs) to differentiate into both astrocytes and microglia in wild-type adult mice using in situ hybridization [60]. Wislet-Gendebien et al. show that nestin-positive (but not nestin-negative) mesenchymal stem cells are able to favor the astroglial lineage in certain stem cell progenitors. They also demonstrate that mesenchymal stem cells express leukemia inhibitory factor (LIF), CNTF, and BMP2 and BMP4 (bone morphogenic protein) mRNAs-cytokines known to play a role in astroglial fate decision [61].

3.2.7 Neoangiogenesis

Further secretion of growth factors like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and brain fibroblast growth factor (bFGF) leads to neoangiogenesis and upregulation of hormones like erythropoietin [62]. The cascade of events triggered due to these leads to formation of new vessels as well as improved blood circulation, thus retrieving lost tissue functions. Stem cells may thus be instrumental in arresting the disease progression through the abovementioned mechanisms.

4. Literature review

4.1 Preclinical studies

A wide variety of preclinical studies show that stem cells migrate to and restore lost function of damaged tissue in ALS/MND. Rodent studies have investigated different cell types such as mouse ES cells differentiated to neurons expressing green fluorescent protein (GFP) under the promoter of the motor neuron (MN)-specific gene *hb9*, mesenchymal stem cells (MSCs), human bone marrow mesenchymal stem cells (hMSCs) obtained from an ALS patient (ALS-hMSCs), human neural stem cells (hNSCs), human cord blood stem cells (HuCB-MNCs), human embryonic stem cell-derived motor neuron progenitors (hMNP), bone marrow cells (BMCs), mesenchymal stromal (stem) cells (MSCs), human umbilical cord blood (MNC-hUCB), human fetal spinal neural stem cells (hNSCs), human iPSC-derived neural progenitors (hiPSNPs), HB1.F3.Olig2 cell (stable immortalized hNSCs encoding the *OLIG2* gene)-derived motor neurons, human amniotic mesenchymal stem cells (hAMSCs), glial-rich neural progenitors derived from human iPSCs, enriched population of embryonic stem cell-derived astrocytes (hES-AS), and neural progenitor cells secreting GDNF (hNPC^{GDNF}) [63–79].

Primarily, stem cells have been shown to have a vast repertoire of paracrine effects, including release of neurotrophic factors such as GDNF, BDNF, vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, NGF, and neurotrophin (NT)-3. Stem cells also confer neuroprotection by migrating, efficiently engrafting into target tissue, reducing astrogliosis, and differentiating into neuroglial cell types. Further, they improve motor performance as measured on rotarod (test measuring rodent balance, grip strength, endurance, and motor coordination), delay disease pathology, and safely extend survival in ALS rodent models (see Appendix) [20, 63–79].

A limitation of preclinical models of ALS, however, is the inherent inability to replicate the *sporadic* onset of ALS/MND, which constitutes majority of patients in clinical scenario [79]. Additionally, an obvious drawback is the underrepresentation of the genomic, anatomical, and physiological complexity of humans by the disease models, which may preclude the translation of results obtained in preclinical settings to the treatment of ALS patients.

4.2 Clinical studies

4.2.1 Worldwide published data

A systematic review and meta-analysis of clinical studies by Moura et al. [80] have suggested that stem cell therapy is a promising therapy and highlighted the need for studies with rigorous methodologies to better understand the efficacy of these therapies. **Table 7** (see Appendix) summarizes the studies reviewed by Moura et al. and other studies that were published using stem cells as therapy in the past decade. Nineteen clinical studies are summarized; a variety of cells have been investigated in clinical settings, such as:

1. Autologous mesenchymal stem cells (intraspinal) [81]
2. Bone marrow-derived hematopoietic progenitor stem cells (intraspinal) [18]
3. Autologous peripheral blood stem (intracerebral) [82]

4. Autologous bone marrow stem cells (intrathecal) [83]
5. Autologous mesenchymal stem cells (intrathecal and intravenous) [31]
6. Olfactory ensheathing cells (intracerebral) and autologous mesenchymal stromal cells (intrathecal and intravenous or only intrathecal) [84]
7. Neural stem cells derived from a fetal spinal cord (intrathecal) [85]
8. Mesenchymal stem cells induced to secrete neurotrophic factors (intramuscular, intrathecal, or both) [86]
9. Autologous bone marrow stem cells (intraspinous) [34]
10. Autologous mesenchymal stem cells (intraspinous) [21]
11. Fetal olfactory ensheathing cells (intracerebral) [19]
12. Fetal-derived neural stem cells (intraspinous) [22]
13. NSI-566RSC (Neuralstem, Inc.), a human neural stem cell (intrathecal) [87]
14. Autologous bone marrow mononuclear cells (intrathecal) [16]
15. Mesenchymal stem cells (intrathecal) [35]
16. Autologous mesenchymal stem cells (intravenous, intrathecal) [88]
17. Autologous bone marrow stem cells (intramedullary) [89]
18. Autologous mesenchymal stem cells (intrathecal) [36]

Overwhelmingly, results point toward a robust safety profile for stem cell treatment in ALS/MND. Stem cell therapy has also proven to be efficacious in mitigating the hostility of a degenerating prognosis in all these studies (see Appendix). These data collectively advocate for the safety and efficacy of various types of stem cells for the treatment of this disease, although small scale; larger clinical trials with sufficient power are required for clearing the turbid field of ALS/MND therapy.

4.2.2 Our published results

4.2.2.1 Our published protocol for stem cell therapy in ALS/MND

4.2.2.1.1 Pre-intervention procedures

We use intrathecal autologous bone marrow mononuclear cell (BMMNC) transplantation for the treatment of ALS/MND, chosen according to the World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects. The ethical approval for the intervention is obtained from Institutional Ethics Committee (IEC). Our exclusion criteria include the presence of respiratory distress; thus, the effect of stem cell therapy on such patients cannot be assessed. Our inclusion criteria involve patients diagnosed as definite or probable ALS according to revised El Escorial criteria [90]. The procedure is

explained to the patients in detail, and a written informed consent is obtained. Patients are thoroughly examined by an experienced team of doctors and therapists. Pre-surgical routine blood tests, urinalysis, and chest X-ray are carried out for assessing anesthetic and surgical fitness. About 300 µg of granulocyte colony-stimulating factor (G-CSF) injections are administrated subcutaneously 48 and 24 hours prior to BMMNC transplantation, as they enhance the mobility of BMMNCs, stimulates CD34⁺ cells, and increases their survival as well as multiplication rate [91]. The transplant is then carried out in three steps (**Figure 4**).

4.2.2.1.2 Bone marrow aspiration

Performed in the operation theater under aseptic conditions, 100–120 ml of bone marrow is aspirated under local anesthesia from the region of anterior superior iliac spine and collected in the heparinized tubes.

4.2.2.1.3 Cell separation

Using density gradient centrifugation, stem cells are separated. The cell pellet is analyzed under a microscope using trypan blue to check for the viability of the cells. Cell number is counted using Tali cell counter. FACS analysis using CD34 PE antibody is used for identification of CD34⁺ cells.

4.2.2.1.4 Cell transplantation

In the operation theater under aseptic conditions, the cells are transplanted intrathecally into the cerebrospinal fluid through lumbar puncture between the level of fourth and fifth lumbar vertebra, using an 18G Touhy needle.

4.2.2.1.5 Posttransplantation

Cell transplantation is followed by standard multidisciplinary rehabilitation including physiotherapy, occupational therapy, speech therapy, psychological intervention, aquatic therapy, and dietary advice. This approach is termed as neuro-regenerative rehabilitative therapy (NRRT). Standard medical treatment was continued with Rilutor. Tablet lithium was prescribed for 6 weeks for its neuroprotective properties. Lithium levels were monitored.

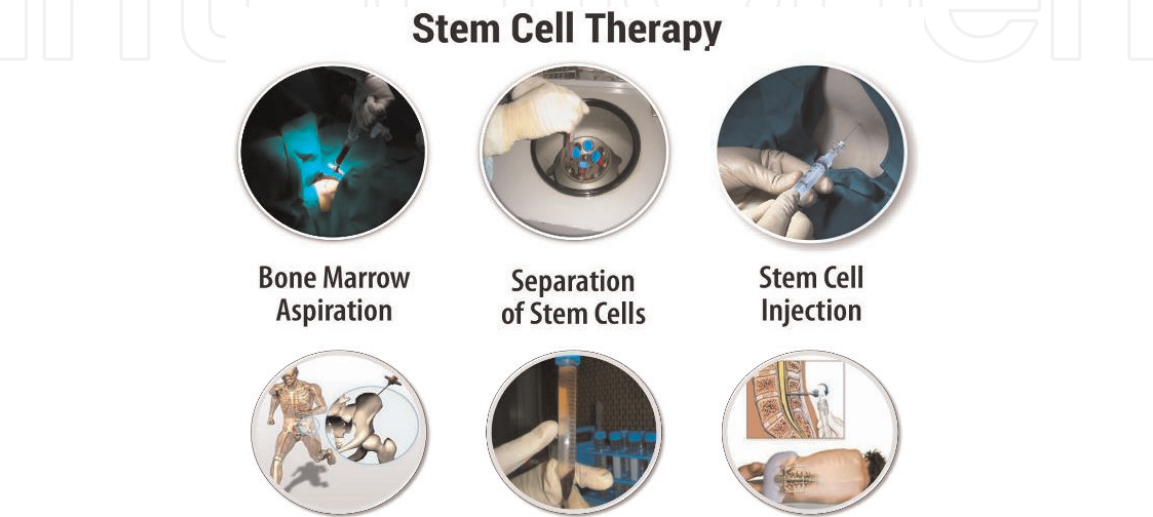


Figure 4.
Stem cell therapy protocol at NeuroGen Brain and Spine Institute.

4.2.2.2 Case series

We have published a retrospective controlled cohort study with a total of 57 ALS patients that investigates the effects of stem cell therapy, in addition to standard rehabilitation, lithium, and riluzole [79]. Out of these, 37 patients underwent autologous BMMNC transplantation, while the remaining 20 did not; these served as controls. We saw that there was a clinically significant difference of 30.38 months between the average survival duration of intervention and control groups. Intervention group survived for 87.76 (10.45) months, while controls survived for 57.38 (5.31) months. Patients with the onset of the disease below 50 years of age survived significantly longer ($p = 0.039$), while limb symptom onset and co-administration of lithium improved survival duration in a clinically significant manner [16]. Lithium increases the survival, potency, and target tissue integration of BMSCs [92]. It has been shown to confer neuroprotection in vitro by enhancing cellular BDNF [93]. In vivo, lithium has been shown to activate autophagy, normalize mitochondrial aberration, and suppress reactive astrogliosis. It also reduces ubiquitinated protein aggregates and increases the number of spared motor neurons in transgenic ALS mice [94]. Further, lithium is well tolerated by ALS patients who are on riluzole, even though it may not be effective by itself for treating ALS. This was confirmed by two trials: a phase III multicenter, randomized, double-blind, placebo-controlled trial (LiCALS) by Al-Chalabi et al. [95] and a phase IIb randomized, double-blind, placebo-controlled, sequential trial by Verstraete et al. [96]. Taken together, these results suggest that a combination strategy of stem cells and lithium may have played a pronounced role in the outcomes of this study, summarizes the prognostic factors that influence survival in the intervention group. Younger age at symptom onset and spinal symptom onset favors longer survival durations according to our findings. Post intervention, lithium prescription combined with standard riluzole treatment and comprehensive rehabilitation enhances the effect of cellular therapy (**Figure 5** and **Table 1**).

4.2.2.3 Case reports

A 40-year-old female suffering from ALS for 3 years was given intrathecal autologous BMMNC transplantation along with riluzole, lithium, and intensive rehabilitation. The disease progression slowed over 17 months along with improvements in neurological symptoms. de Carvalho et al. have previously reported that the ALSFRS-R score deteriorates about 17% every 6 months [97]; here, the ALSFRS-R score dropped only by 8% over 17 months after cell transplantation (**Figure 6** and **Table 2**) [98].

A 41-year-old female suffering from ALS for 3 years was given intrathecal autologous BMMNC therapy combined with riluzole, neuro-rehabilitation, and 6 weeks of lithium. Her ALSFRS-R score increased from 29 to 32, and FIM score increased from 48 to 64. The highlight of this case is halting of disease progression with symptomatic improvements over a period of 12 months after intervention (**Table 3** and **Figure 7**).

A 63-year-old man who underwent autologous intrathecal BMMNC transplantation as a therapy in a clinical case of MND followed by multidisciplinary neurorehabilitation showed improvements in muscle strength, fine motor activities, fasciculation, cramps, and walking (**Table 4**). ALSFRS-R score improved from 33 to 37; Berg's balance score improved from 43 to 50, and 6-minute walk test improved from 283.8 to 303.6 m. His FIM score remained unchanged at 113. These improvements may be attributed to cellular therapy along with standard treatment and neurorehabilitation [99].

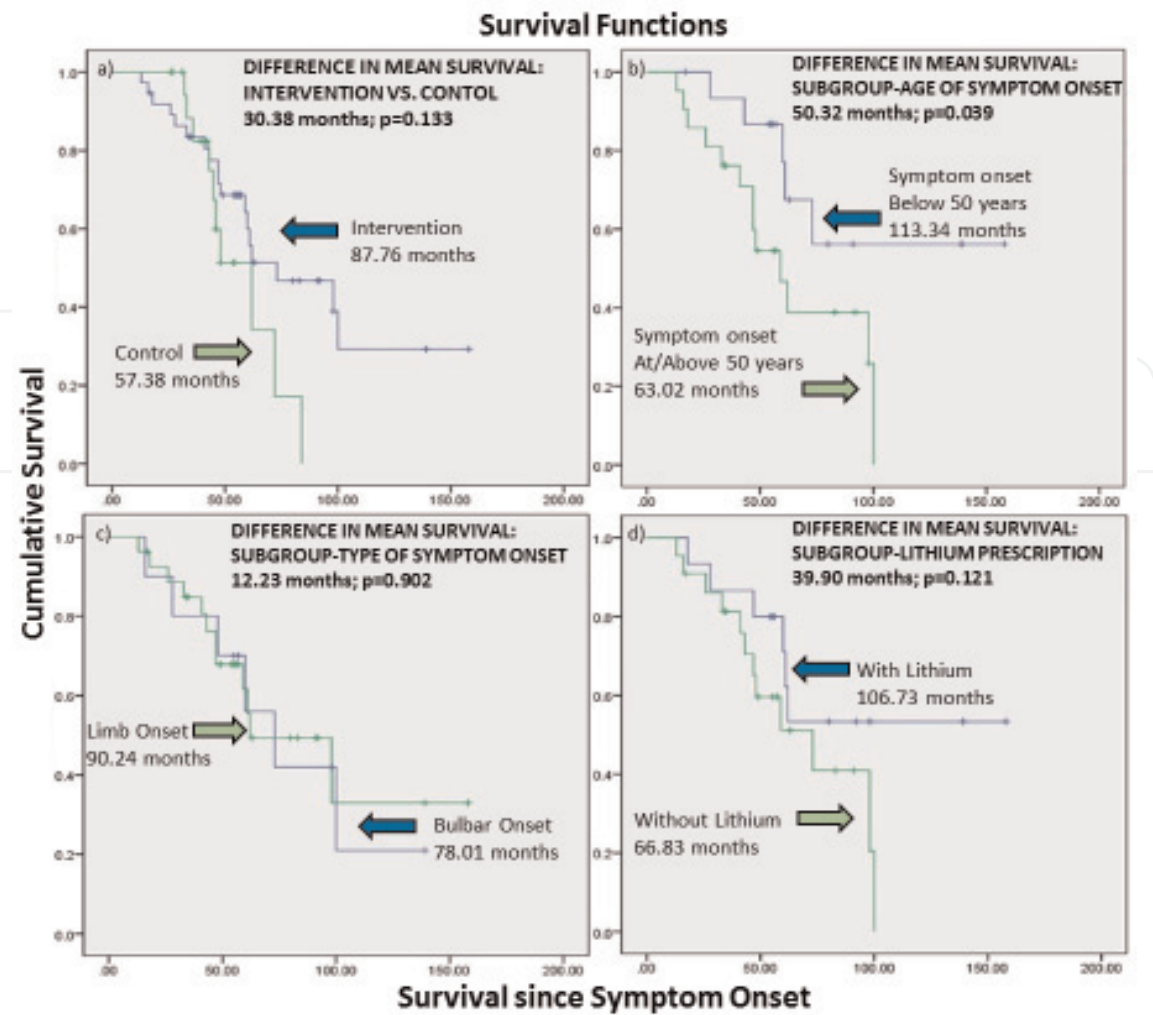


Figure 5.
(a) Kaplan-Meier survival analysis comparing the mean survival duration of the intervention ($n = 37$) and control group ($n = 20$) from Sharma et al. [16]. Mean survival duration of patients in the intervention group was higher than the control group by a clinically significant difference of 30.38 months. (b) Subgroup analysis of the effect of age of symptom onset on survival duration within the intervention group, from Sharma et al. [16] shows significantly higher survival of those with an onset of symptoms above 50 years of age ($p = 0.039$). (c) Subgroup analysis of the effect of the type of symptom onset (limb vs. bulbar) on survival duration in the intervention group shows higher survival of patients with limb onset of symptoms by 12.23 months. (d) Subgroup analysis of the effect of lithium prescription on survival duration within the intervention group shows a clinically higher survival of 106.73 months of the group prescribed with lithium. This is a clinically significant difference of 30.90 months as compared to controls, whose average survival was 66.83 months.

Prognostic factor		Median survival since symptom onset	p-Value
Lithium	Given	106.73 (15.69)	0.121
	Not given	66.83 (7.52)	
Age at symptom onset	Below 50 years	113.34 (15.45)	0.039*
	Above 50 years	63.02 (7.7)	
Type of symptom onset	Limb	78.01 (14.23)	0.902
	Bulbar	90.24 (13.27)	

*Statistically significant ($p < 0.05$).

Table 1.
Summary of prognostic factors affecting patient survival in the intervention group, from Sharma et al. [16]. Standard deviation is indicated in parentheses.

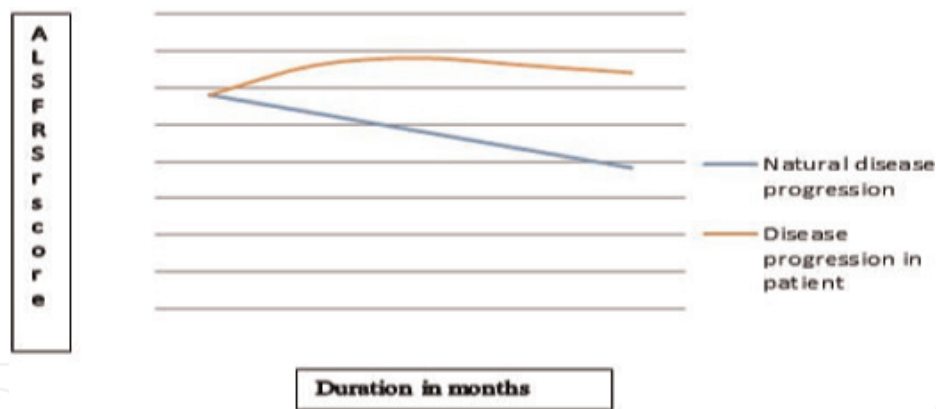


Figure 6. Maintenance of a 40-year-old female ALS patient's condition over the period of 17 months. Disease progression in patient (red) was compared using ALSFRS-R with average values obtained from de Carvalho et al. (blue) at 0, 5, 15, and 17 months [97].

Outcome measures	At assessment before the first transplant	At 5 months after the first transplant (just before the second transplant)	At 15 months after the first transplant	At 17 months after the first transplant
ALSFRS-R	36	36	36	33
FIM	113	113	113	113

Table 2. ALSFRS-R and FIM instrument scores of the patient remain stable as compared to assessment, when followed up at 5, 15, and 17 months.

Outcome measures	At assessment before the first transplant	At 2 months after the first transplant	At 6 months after the first transplant	At 9 months after the first transplant	At 12 months after the first transplant
ALSFRS-R	29	33	34	33	32
FIM	48	72	73	71	64

Table 3. ALSFRS-R and FIM scores of a 41-year-old female ALS patient measured at assessment and after intervention (at 2, 6, 9, and 12 months post autologous BMMNC transplantation) depict stark improvement.

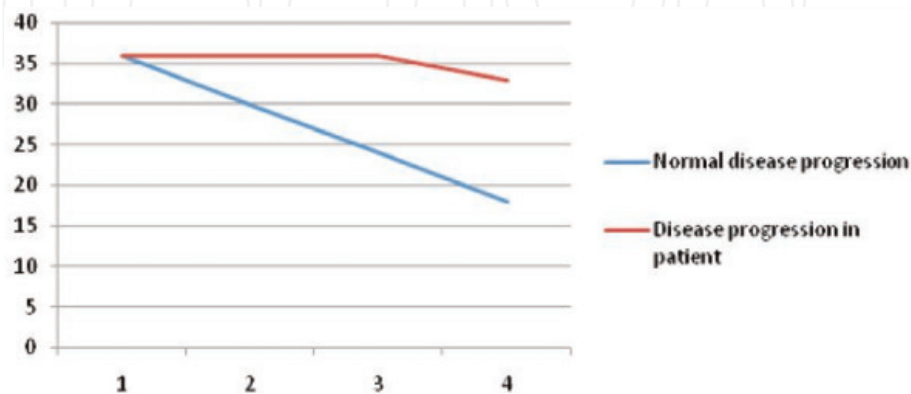


Figure 7. ALSFRS-R and FIM scores were marked at 2, 4, 6, 9, and 12 months for this patient. ALSFRS-R score was measured across survival duration (in months) to compare the disease progression of this patient with natural disease progression in ALS, as measured by de Carvalho et al. [97]. The patient's condition was maintained over a period of 12 months.

Outcome measures	Pre-first SCT	At 6 months post the first SCT
ALSFRS-R	33	37
FIM	113	113
6-minute walk test	283.8 m	303.6 m
Berg's balance score	43	50

Table 4.
Improvements in outcome measures over a period of 6 months in a 63-year-old male ALS patient. ALSFRS-R, FIM, and 6-minute walk test were measured. Although not standard, Berg's balance test was administered to assess his balance.

A 29-year-old female patient with anterior horn cell involvement suffering from MND for 5 years presented with complaints of sudden onset of weakness in bilateral lower limbs post pregnancy. Her progressive condition was followed by gradual involvement of upper limbs also; EMG studies were also suggestive of MND. Her features were suggestive of pure motor system involvement affecting lower motor neurons. Post NRRT, she immediately showed clinical and functional improvements [17].

4.2.2.4 Unpublished data

4.2.2.4.1 Female hormones enhance the neuroprotective benefits of cellular transplantation in patients with amyotrophic lateral sclerosis (ALS)

The male/female ratio in ALS/MND worldwide points toward a lower incidence in females as compared to males. We hypothesized that this was due to a neuroprotective effect conferred by female hormones. In order to investigate this hypothesis, we designed a cohort study with 40 sequentially recruited ALS patients (28 males and 12 females) who were treated with stem cell therapy. To study the effect of reproductive hormones, patients were divided into pre- and postmenopausal women and men below and above 50 years of age.

We saw that percentage survival was highest in the premenopausal women (100%) followed by men below the age of 50 years (75%), postmenopausal women (60%), and men above the age of 50 years (45%). The disease progression was also slowest in the premenopausal women, followed by postmenopausal women, and men below 50 years of age; it was fastest in men above the age of 50 years (Table 5).

Group	Mean pre-ALSFRS-R	Mean post-ALSFRS-R	Difference	Average follow-up (months)	Percentage mortality
Premenopausal females (7)	26	23	3	20	0
Postmenopausal females (5)	21	14	7	14	40
Males below 50 years of age (16)	25	20	5	10	25
Males above 50 years of age (12)	32	25	7	13	55

Table 5.
Percentage mortalities across the four subgroups in a cohort study.

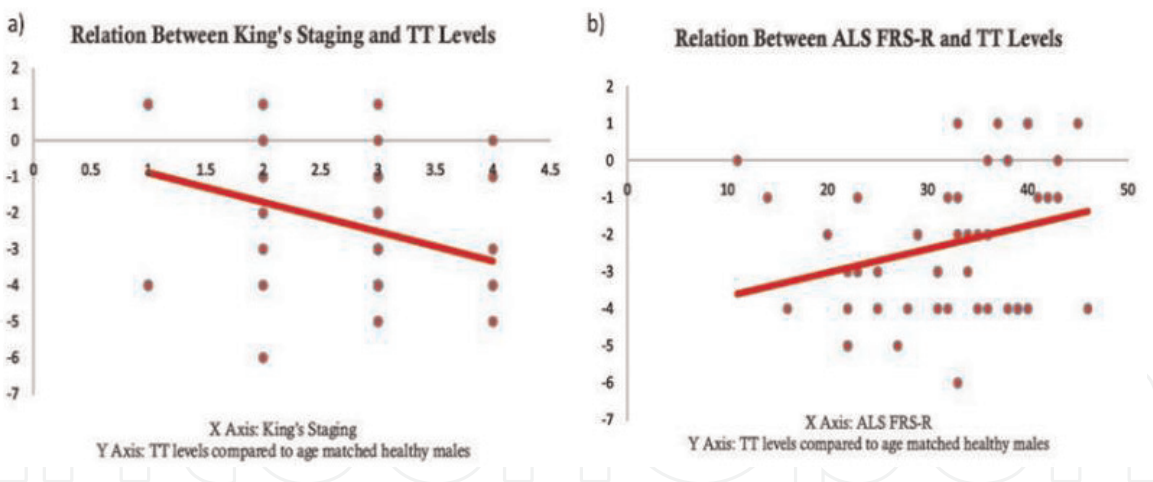


Figure 8.
Relationship of testosterone with (a) King's staging and (b) ALSFRS-R.

4.2.2.4.2 Correlation of testosterone levels with progression of amyotrophic lateral sclerosis: a cross-sectional study

We conducted an open-label nonrandomized cross-sectional study to interrogate the relationship of testosterone (TT) with disease progression. We found that 39 of the total 50 (78%) ALS patients had plasma TT levels lower than the mean levels in healthy, age-matched control males. We also found a decline in TT levels as the disease progressed on King's staging as well as ALSFRS-R (**Figure 8**). There was a statistically significant moderate monotonic correlation between ALSFRS-R scores and King's staging of patients with plasma testosterone levels (ALSFRS-R: $r = +0.33$;

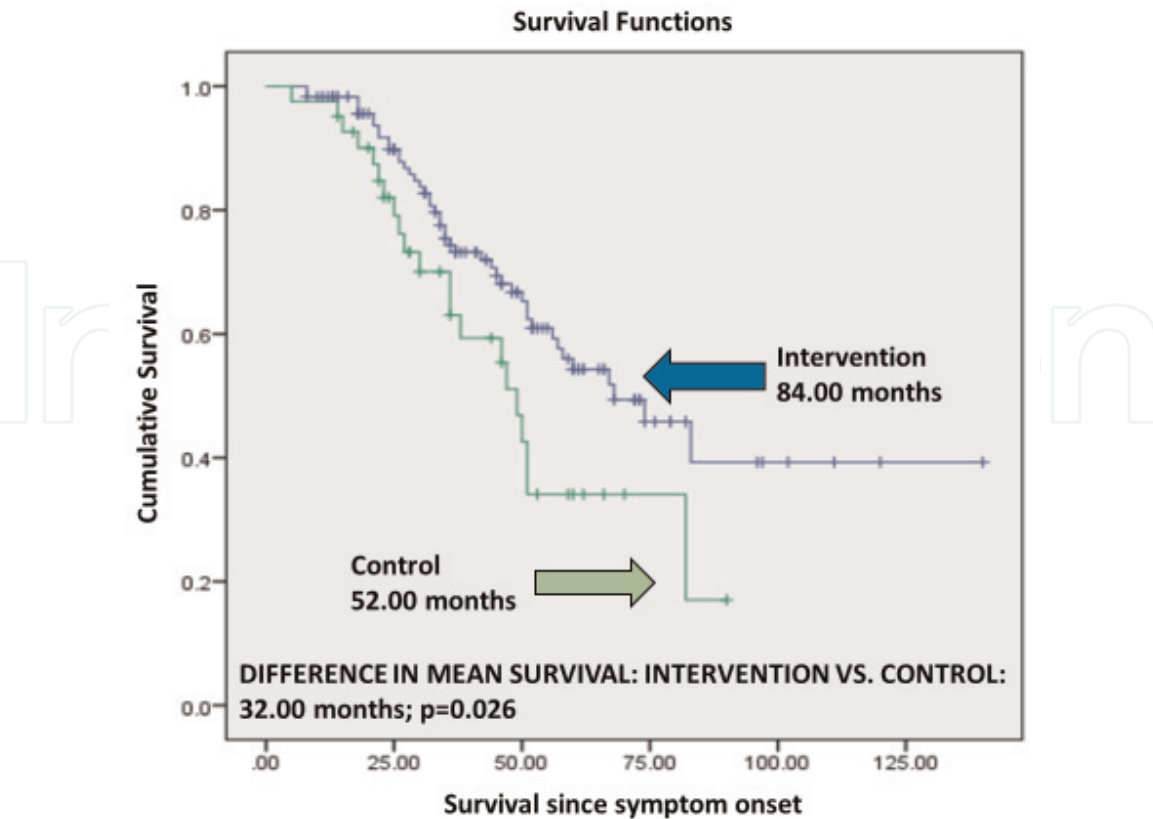


Figure 9.
Kaplan-Meier survival curves for control vs. intervention groups in our open-label study with a total of 157 subjects (intervention = 116; control = 41). On an average, the treated group survived for 32 months longer than the control group ($p = 0.026$).

King’s staging; $r = -0.35$; $p = 0.01$). Taken together, these results suggest that reduced testosterone may exacerbate motor neuron loss or cause other etiopathological dysfunctions that remain to be elucidated.

4.2.2.4.3 Effect of intrathecal application of autologous bone marrow mononuclear cells on survival duration in ALS

We performed a large, open-label cohort study, which included 157 patients diagnosed with ALS from September 2013 to May 2017. About 116 patients who received cell transplantation (autologous BMMNCs) together with standard treatment formed the treatment group, and 41 patients who received only the standard treatment formed the control group (**Figure 9** and **Table 6**). We observed that on an average, the treated group survived for 32 months longer than the control group ($p = 0.026$).

Collectively, these findings were indicative of possible neuroprotective benefits of female reproductive hormones. Prognostic factors that predict improved survival and retarded disease progression include lithium co-administration, limb onset of symptoms, younger age of symptom onset, and, most importantly, presence of female hormones (**Figure 10**).

Characteristics	Intervention		Control
Total number of patients	157		41
Gender	Males	81	30
	Females	35	11
Mean age at symptom onset (years)	51 ± 11		53 ± 9
Type of symptom onset	Bulbar	26	6
	Limb	90	21

Table 6. Demographic data for an open-label study with 157 participants.

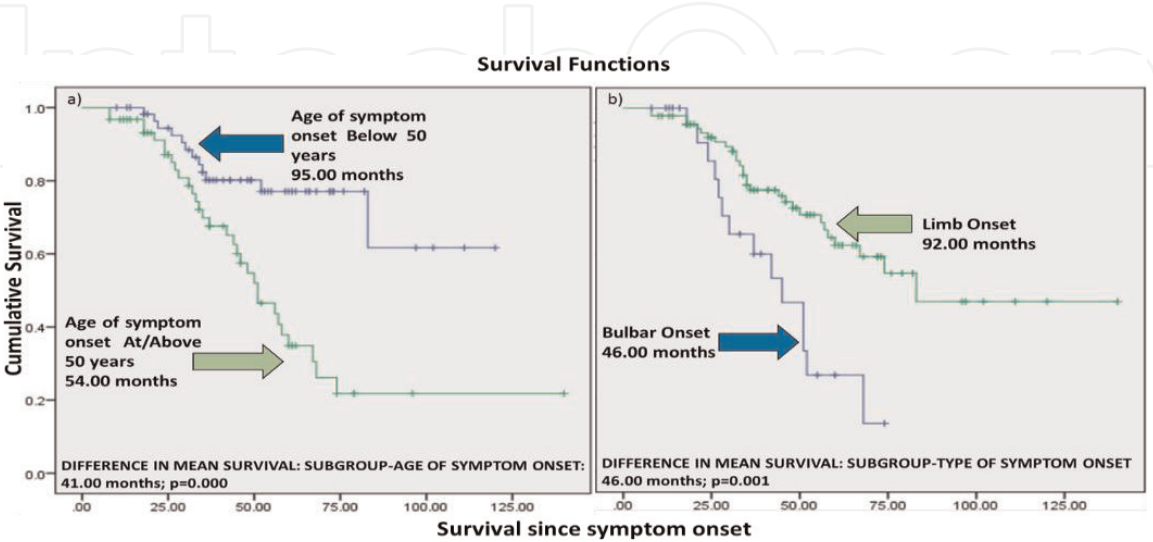


Figure 10. (a) Subgroup analysis: survival duration was 46 months more in patients with limb onset as compared to bulbar onset ($p = 0.001$). Bulbar onset $n = 26$ patients and limb onset $n = 90$. (b) Survival duration was 41 months more in patients with age of symptom onset < 50 years as compared to symptom onset at/above 50 years ($p < 0.000$).

5. Conclusion and future directions

Stem cell therapy is a novel, promising modality for the treatment of ALS/MND. Robust safety profiles, low risk-to-benefit ratio, and ease of access make this approach a strong contender in the race against ALS/MND. Consistently, autologous BMMNC therapy has been proven to mitigate disease progression; however, this response may be dependent on various factors, like age of symptom onset, gender, hormones, type of onset (limb vs. bulbar), and genetic makeup of the patient, to name a few. Younger patients—especially premenopausal females—may respond better to autologous BMMNC therapy. Stem cells combat tissue degeneration via a host of somatic and paracrine mechanisms, including neurogenesis, astrogliogenesis, neoangiogenesis, and immunomodulation. Multidisciplinary neurorehabilitation enhances the response to cellular therapy.

Although not a cure yet, a combinatorial approach integrating stem cell therapy, intensive neurorehabilitation, and current pharmacotherapeutic agents (e.g., riluzole, lithium, etc.) may be the best way forward. Studies that interrogate the genetics of patients and their family are the need of the hour, to enhance response to treatment and develop diagnostics and biomarkers. Also, the role of reproductive hormones such as progesterone, estradiol, and testosterone needs to be further explored. Larger clinical studies with stringent criteria are required to understand the efficacy of these combined methods in the treatment of ALS/MND. The current scenario suggests that autologous stem cell therapy can be considered along with standard treatment in carefully selected patients of ALS/MND.

Conflict of interest

The authors declare no conflict of interest in the writing of this chapter.

Appendix

See Table 7.

Authors, year, country (type of study, sample size)	Type of cells used (route of administration)	Results
Mazzini et al. [81], Italy (clinical trial, 7)	Autologous mesenchymal stem cells (intraspinal)	Slowing down of the linear decline of muscular strength was evident in four patients and improvement in strength in two patients in proximal lower limb muscles was observed
Deda et al. [18], Turkey (clinical trial, 13)	Bone marrow-derived hematopoietic progenitor stem cells (intraspinal)	9/13 (69.23%) patients improved as compared with their preoperative status, as confirmed by EMG
Martinez et al. [82], Mexico (controlled clinical trial, 10)	Autologous peripheral blood stem (intracerebral)	The survival of treated patients was statistically higher ($p = 0.01$) than untreated control patients
Prabhakar et al. [83], India (clinical trial—pilot study, 10)	Autologous bone marrow stem cells (intrathecal)	There was no significant deterioration in ALSFRS-R composite score from baseline at a 1-year follow-up ($p = 0.090$). The median survival post procedure was 18.0 months and median time to 4-point deterioration was 16.7 months

Authors, year, country (type of study, sample size)	Type of cells used (route of administration)	Results
Karussis et al. [31], Israel (phase I/phase II clinical trial, 19)	Autologous mesenchymal stem cells (intrathecal and intravenous)	The mean ALSFRS-R score remained stable during the first 6 months of observation. In ~80% of the patients, FVC values remained stable or above 70% for a time of 9 months and remained in ~60% of patients at 12 months after application. Signs of disease stabilization in some patients during the first 6 months after the intervention
Gamez et al. [84], Spain (observational study, 12)	Olfactory ensheathing cells (intracerebral) and autologous mesenchymal stromal cells (intrathecal + intravenous or only intrathecal)	No changes in the decline of FVC and ALSFRS-R compared with the disease's natural history were observed
Riley et al. [85], USA (phase I safety trial, 12)	Neural stem cells derived from a fetal spinal cord (intrathecal)	Procedural safety of unilateral and bilateral intraspinal lumbar microinjections has been suggested by the results of this trial
Petrou et al. [86], Israel (open-label proof-of-concept study, phase I/phase II and IIa)	Mesenchymal stem cells induced to secrete neurotrophic factors (intramuscular (IM), intrathecal (IT) or (IT+IM))	Progression rate of the ALSFRS-R score in the IT (or IT+IM)-treated patients was reduced from -1.2 to 0.6 ALSFRS-R points/month ($p = 0.052$), and the progression rate of the forced vital capacity reduced from -5.1% to -1.2%/month during the 6 months follow-up vs. pretreatment period
Blanquer et al. [34], Spain (clinical trial, pilot safety study, 11)	Autologous bone marrow stem cells (intrapinal)	7/11 (63.63%) patients remained stable post procedure
Mazzini et al. [21], Italy (clinical trial, long-term safety study, 9)	Autologous mesenchymal stem cells (intrapinal)	Brain MRI revealed no structural changes relative to baseline throughout follow-up. No deterioration noted in the psychosocial status as well
Huang et al. [19], China (controlled pilot study, 35)	Fetal olfactory ensheathing cells (intracerebral)	7/14 improved; 2/14 remained stable compared to the entry in the treated group, while only 1/17 of the patients remained stable within control
Glass et al. [22], USA (phase I clinical trial, 12)	Fetal neural stem cells (intrapinal)	Patients remained stable and tolerated the therapy well, as seen in clinical assessments at 6–18 months
Riley et al. [87], USA (phase I trial, 15)	NSI-566RSC-a human neural stem cell (intrathecal)	Cellular delivery to the cervical or thoracolumbar spinal cord was well tolerated by the patients
Sharma et al. [16], India (retrospective controlled cohort study, 57)	Autologous bone marrow mononuclear cells (intrathecal)	Mean survival duration of intervention was 87.76 months, which was higher than the control (57.38 months) or previous epidemiological studies. Survival duration was significantly ($p = 0.039$) higher in people with

Authors, year, country (type of study, sample size)	Type of cells used (route of administration)	Results
		the onset of the disease below 50 years of age. Limb onset and lithium also showed positive influence on the survival duration
Oh et al. [35], South Korea (single open-label phase I clinical trial, 8)	Mesenchymal stem cells (intrathecal)	Decline in the ALSFRS-R score was slow during the 6-month follow-up period
Rushkevich et al. [88], Belarus (controlled clinical trial, 10)	Autologous mesenchymal stem cells (intravenous, intrathecal)	Evaluation of the 12-month follow-up revealed slowing down of the disease progression in 10 patients
Ruiz-López et al. [89], Spain (phase I clinical trial, 11)	Autologous bone marrow stem cells (intramedullary)	All 11 patients were 100% stable
Syková et al. [36], Czech Republic (phase I/phase IIa prospective, nonrandomized, open-label clinical trial, 26)	Autologous mesenchymal stem cells (intrathecal)	A significant reduction/stabilization was found in ALSFRS-R decline at 3, 6, 9, and 12 months after treatment
<i>All studies demonstrate safety of stem cells, except Glass et al. [22]. Safety in this table does not describe events unrelated to stem cell therapy.</i>		

Table 7.
Significant clinical studies employing stem cell therapy for treatment of ALS/MND, primarily from March 2009 to February 2019.

Author details

Alok Sharma¹, Hemangi Sane², Nandini Gokulchandran³, Prerna Badhe⁴,
Amruta Paranjape^{2,5*}, Radhika Pradhan², Rohit Das⁵ and Hema Biju⁵

1 NeuroGen Brain and Spine Institute, Navi Mumbai, India

2 Department of Research and Development, NeuroGen Brain and Spine Institute, Navi Mumbai, India

3 Department of Medical Services and Clinical Research, NeuroGen Brain and Spine Institute, Navi Mumbai, India

4 Department of Regenerative Laboratory Services, NeuroGen Brain and Spine Institute, Navi Mumbai, India

5 Department of Neurorehabilitation, NeuroGen Brain and Spine Institute, Navi Mumbai, India

*Address all correspondence to: publications@neurogen.in

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Talbot K. Motor neuron disease: The bare essentials. *Practical Neurology*. 2009;**9**(5):303-309
- [2] McDermott CJ, Shaw PJ. Diagnosis and management of motor neurone disease. *BMJ*. 2008;**336**(7645):658-662
- [3] Marin B, Boumédiène F, Logroscino G, Couratier P, Babron M-C, Leutenegger AL, et al. Variation in worldwide incidence of amyotrophic lateral sclerosis: A meta-analysis. *International Journal of Epidemiology*. 2017;**46**(1):57-74
- [4] Manjaly ZR, Scott KM, Abhinav K, Wijesekera L, Ganesalingam J, Goldstein LH, et al. The sex ratio in amyotrophic lateral sclerosis: A population based study. *Amyotrophic Lateral Sclerosis: Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases*. 2010;**11**(5):439-442
- [5] Mitchell JD, Callaghan P, Gardham J, Mitchell C, Dixon M, Addison-Jones R, et al. Timelines in the diagnostic evaluation of people with suspected amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND)—A 20-year review: Can we do better? *Amyotrophic Lateral Sclerosis: Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases*. 2010;**11**(6):537-541
- [6] Jenkins TM, Hollinger H, McDermott CJ. The evidence for symptomatic treatments in amyotrophic lateral sclerosis. *Current Opinion in Neurology*. Oct 2014;**27**(5):524-531
- [7] Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database of Systematic Reviews*. 2002;**2**:CD001447
- [8] Ikeda K, Iwasaki Y. Edaravone, a free radical scavenger, delayed symptomatic and pathological progression of motor neuron disease in the wobbler mouse. *PLoS ONE*. 2015;**10**(10):e0140316
- [9] Cruz MP. Nuedexta for the treatment of pseudobulbar affect. *Pharmacology and Therapeutics*. 2013;**38**(6):325-328
- [10] Hardiman O. Multidisciplinary care in ALS: Expanding the team. *Amyotrophic Lateral Sclerosis*. 2012;**13**(2):165-165
- [11] O'Brien M, Whitehead JB, Douglas Mitchell J. Multidisciplinary team working in motor neurone disease: Patient and family carer views. *British Journal of Neuroscience Nursing*. 2011;**7**(4):580-585
- [12] Paganoni S, Karam C, Joyce N, Bedlack R, Carter GT. Comprehensive rehabilitative care across the spectrum of amyotrophic lateral sclerosis. *NeuroRehabilitation*. 2015;**37**(1):53-68
- [13] Sharma A, Gokulchandran N, Sane H, Nagrajan A, Paranjape A, Kulkarni P, et al. Autologous bone marrow mononuclear cell therapy for autism: An open label proof of concept study. *Stem Cells International*. 2013;**2013**:623875
- [14] Chahine NA, Wehbe T, Rashed J, Hilal R, Elias N. Autologous bone marrow derived stem cells for the treatment of multiple sclerosis. *International Journal of Stem Cells*. 2016;**9**(2):207-212
- [15] Sharma A, Sane H, Badhe P, Gokulchandran N, Kulkarni P, Lohiya M, et al. A clinical study shows safety and efficacy of autologous bone marrow mononuclear cell therapy to improve quality of life in muscular dystrophy patients. *Cell Transplantation*. 2013;**22**(1_suppl):127-138
- [16] Sharma AK, Sane HM, Paranjape AA, Gokulchandran N, Nagrajan A,

D'sa M, et al. The effect of autologous bone marrow mononuclear cell transplantation on the survival duration in amyotrophic lateral sclerosis—A retrospective controlled study. *American Journal of Stem Cells*. 2015;4(1):50-65

[17] Sharma A, Badhe P, Shetty O, Vijaygopal P, Gokulchandran N, Jacob VC, et al. Autologous bone marrow derived stem cells for motor neuron disease with anterior horn cell involvement. *The Bombay Hospital Journal*. 2011;53(1):71-75

[18] Deda H, Inci MC, Kürekçi AE, Sav A, Kayihan K, Özgün E, et al. Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: A 1-year follow-up. *Cytotherapy*. 2009;11(1):18-25

[19] Huang H, Chen L, Xi H, Wang H, Zhang J, Zhang F, et al. Fetal olfactory ensheathing cells transplantation in amyotrophic lateral sclerosis patients: A controlled pilot study. *Clinical Transplantation*. 2008;22(6):710-718

[20] Mazzini L, Gelati M, Profico DC, Sgaravizzi G, Progetti Pensi M, Muzi G, et al. Human neural stem cell transplantation in ALS: Initial results from a phase I trial. *Journal of Translational Medicine*. 2015;13:17

[21] Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S, et al. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: A long-term safety study. *Cytotherapy*. 2012;14(1):56-60

[22] Glass JD, Boulis NM, Johe K, Rutkove SB, Federici T, Polak M, et al. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: Results of a phase I trial in 12 patients. *Stem cells (Dayton, Ohio)*. 2012;30(6):1144-1151

[23] Stem Cell Basics II. stemcells.nih.gov [Internet]. Available from: [https://](https://stemcells.nih.gov/info/basics/2.htm)

stemcells.nih.gov/info/basics/2.htm [Accessed: 16 October 2018]

[24] Marędziak M, Śmieszek A, Chrzastek K, Basinska K, Marycz K. Physical activity increases the total number of bone-marrow-derived mesenchymal stem cells, enhances their osteogenic potential, and inhibits their adipogenic properties. *Stem Cells International*. 2015;2015:11. Article ID: 379093

[25] Weissman IL. Stem cells: Units of development, units of regeneration, and units in evolution. *Cell*. 2000;100(1):157-168

[26] Morrison SJ, Spradling AC. Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008;132(4):598-611

[27] Khademhosseini A, Karp JM, Gerecht-Nir S, Ferreira L, Annabi N, Sirabella D, et al. Chapter 32—Embryonic stem cells as a cell source for tissue engineering. In: Lanza R, Langer R, Vacanti J, editors. *Principles of Tissue Engineering*. 4th ed. Boston: Academic Press; 2014. pp. 609-638. Available from: <http://www.sciencedirect.com/science/article/pii/B978012398358900032X> [cited 8 October 2018]

[28] Stem Cell Basics V. stemcells.nih.gov [Internet]. Available from: <https://stemcells.nih.gov/info/basics/5.htm> [Accessed: 16 October 2018]

[29] Gage FH. Mammalian neural stem cells. *Science*. 2000;287(5457):1433-1438

[30] O'Donoghue K, Fisk NM. Fetal stem cells. *Best Practice & Research. Clinical Obstetrics & Gynaecology*. 2004;18(6):853-875

[31] Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, et al. Safety and

- immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. *Archives of Neurology*. 2010;**67**(10):1187-1194
- [32] Thurairajah K, Broadhead M, Balogh Z, Thurairajah K, Broadhead ML, Balogh ZJ. Trauma and stem cells: Biology and potential therapeutic implications. *International Journal of Molecular Sciences*. 2017;**18**(3):577
- [33] Nagpal A, Choy FC, Howell S, Hillier S, Chan F, Hamilton-Bruce MA, et al. Safety and effectiveness of stem cell therapies in early-phase clinical trials in stroke: A systematic review and meta-analysis. *Stem Cell Research & Therapy*. Aug 30 2017;**8**(1):191
- [34] Blanquer M, Moraleda JM, Iniesta F, Gómez-Espuch J, Meca-Lallana J, Villaverde R, et al. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: A pilot safety study. *Stem cells (Dayton, Ohio)*. 2012;**30**(6):1277-1285
- [35] Oh K-W, Moon C, Kim HY, Oh S-I, Park J, Lee JH, et al. Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. *Stem Cells Translational Medicine*. 2015; **4**(6):590-597
- [36] Syková E, Rychmach P, Drahorádová I, Konrádová Š, Růžicková K, Voříšek I, et al. Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: Results of phase I/IIa clinical trial. *Cell Transplantation*. 2017;**26**(4):647-658
- [37] Waller-Wise R. Umbilical cord blood: Information for childbirth educators. *The Journal of Perinatal Education*. 2011;**20**(1):54-60
- [38] Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS ONE*. 2014;**9**(12):e115963
- [39] Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering*. 2001;**7**(2):211-228
- [40] Lin G, Garcia M, Ning H, Banie L, Guo Y-L, Lue TF, et al. Defining stem and progenitor cells within adipose tissue. *Stem Cells and Development*. 2008;**17**(6):1053-1063
- [41] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; **126**(4):663-676
- [42] de Souza FT, de Souza FC, Mencialha AL. Human induced pluripotent stem cells from basic research to potential clinical applications in cancer. *BioMed Research International*. 2013;**2013**:11 (Article ID 430290)
- [43] Blasco H, Mavel S, Corcia P, Gordon PH. The glutamate hypothesis in ALS: Pathophysiology and drug development. *Current Medicinal Chemistry*. 2014; **21**(31):3551-3575
- [44] Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. 2010;**140**(6): 918-934
- [45] Knott AB, Bossy-Wetzel E. Nitric oxide in health and disease of the nervous system. *Antioxidants & Redox Signaling*. 2009;**11**(3):541-553
- [46] Smith EF, Shaw PJ, De Vos KJ. The role of mitochondria in amyotrophic lateral sclerosis. *Neuroscience Letters*. Jun 30 2017;**pii: S0304-3940(17)30544-X**

- [47] Armon C. From snow to hill to ALS: An epidemiological odyssey in search of ALS causation. *Journal of the Neurological Sciences*. 2018;**391**:134-140
- [48] Al-Chalabi A, Hardiman O. The epidemiology of ALS: A conspiracy of genes, environment and time. *Nature Reviews. Neurology*. 2013;**9**(11):617-628
- [49] Pokrishevsky E, Grad LI, Yousefi M, Wang J, Mackenzie IR, Cashman NR. Aberrant localization of FUS and TDP43 is associated with misfolding of SOD1 in amyotrophic lateral sclerosis. *PLoS ONE*. 2012;**7**(4):e35050
- [50] Keller BA, Volkening K, Droppelmann CA, Ang LC, Rademakers R, Strong MJ. Co-aggregation of RNA binding proteins in ALS spinal motor neurons: Evidence of a common pathogenic mechanism. *Acta neuropathologica*. 2012;**124**(5):733-747
- [51] Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, et al. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*. 2010; **465**(7295):223-226
- [52] Phruksaniyom C, Dharmasaroja P, Issaragrisil S. Bone marrow non-mesenchymal mononuclear cells induce functional differentiation of neuroblastoma cells. *Experimental Hematology & Oncology*. 2013;**2**:9
- [53] Bhasin A, Srivastava MVP, Mohanty S, Vivekanandhan S, Sharma S, Kumaran S, et al. Paracrine mechanisms of intravenous bone marrow-derived mononuclear stem cells in chronic ischemic stroke. *Cerebrovascular Diseases EXTRA*. 2016;**6**(3):107-119
- [54] Pastor D, Viso-León MC, Jones J, Jaramillo-Merchán J, Toledo-Aral JJ, Moraleda JM, et al. Comparative effects between bone marrow and mesenchymal stem cell transplantation in GDNF expression and motor function recovery in a motor neuron degenerative mouse model. *Stem Cell Reviews*. 2012;**8**(2):445-458
- [55] Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 2001;**104**(9):1046-1052
- [56] Kim SH, Oh K-W, Jin HK, Bae J-S. Immune inflammatory modulation as a potential therapeutic strategy of stem cell therapy for ALS and neurodegenerative diseases. *BMB Reports*. 2018;**51**(11):545-546
- [57] Gu R, Hou X, Pang R, Li L, Chen F, Geng J, et al. Human adipose-derived stem cells enhance the glutamate uptake function of GLT1 in SOD1(G93A)-bearing astrocytes. *Biochemical and Biophysical Research Communications*. 2010;**393**(3):481-486
- [58] Mezey É, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow. *Science*. 2000;**290**(5497):1779-1782
- [59] Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis J. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. *GLIA*. 2001;**35**(1):26-34
- [60] Eglitis MA, Mezey É. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**(8):4080-4085
- [61] Wislet-Gendebien S, Bruyère F, Hans G, Leprince P, Moonen G, Rogister B. Nestin-positive mesenchymal stem cells favour the astroglial lineage in

neural progenitors and stem cells by releasing active BMP4. *BMC Neuroscience*. 2004;**5**:33

[62] Xu L, Yan J, Chen D, Welsh AM, Hazel T, Johe K, et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation*. 2006;**82**(7):865

[63] López-González R, Kunckles P, Velasco I. Transient recovery in a rat model of familial amyotrophic lateral sclerosis after transplantation of motor neurons derived from mouse embryonic stem cells. *Cell Transplantation*. 2009;**18**(10–11):1171–1181

[64] Boucherie C, Schäfer S, Lavand'homme P, Maloteaux J-M, Hermans E. Chimerization of astroglial population in the lumbar spinal cord after mesenchymal stem cell transplantation prolongs survival in a rat model of amyotrophic lateral sclerosis. *Journal of Neuroscience Research*. 2009;**87**(9):2034–2046

[65] Kim H, Kim HY, Choi MR, Hwang S, Nam K-H, Kim H-C, et al. Dose-dependent efficacy of ALS-human mesenchymal stem cells transplantation into cisterna magna in SOD1-G93A ALS mice. *Neuroscience Letters*. 2010;**468**(3):190–194

[66] Xu L, Shen P, Hazel T, Johe K, Koliatsos VE. Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. *Neuroscience Letters*. 2011;**494**(3):222–226

[67] Bigini P, Veglianesi P, Andriolo G, Cova L, Grignaschi G, Caron I, et al. Intracerebroventricular administration of human umbilical cord blood cells delays disease progression in two murine models of motor neuron degeneration. *Rejuvenation Research*. 2011;**14**(6):623–639

[68] Wyatt TJ, Rossi SL, Siegenthaler MM, Frame J, Robles R, Nistor G, et al. Human motor neuron progenitor transplantation leads to endogenous neuronal sparing in 3 models of motor neuron loss. *Stem Cells International*. May 23 2011;**2011**(2011):207230

[69] Lee JC, Seong J, Kim SH, Lee SJ, Cho YJ, An J, et al. Replacement of microglial cells using clodronate liposome and bone marrow transplantation in the central nervous system of SOD1(G93A) transgenic mice as an in vivo model of amyotrophic lateral sclerosis. *Biochemical and Biophysical Research Communications*. 2012;**418**(2):359–365

[70] Uccelli A, Milanese M, Principato MC, Morando S, Bonifacino T, Vergani L, et al. Intravenous mesenchymal stem cells improve survival and motor function in experimental amyotrophic lateral sclerosis. *Molecular medicine (Cambridge, Mass.)*. 2012;**18**:794–804

[71] Garbuzova-Davis S, Rodrigues MCO, Mirtyl S, Turner S, Mitha S, Sodhi J, et al. Multiple intravenous administrations of human umbilical cord blood cells benefit in a mouse model of ALS. *PLoS ONE*. Feb 3 2012;**7**(2):e31254

[72] Hefferan MP, Galik J, Kakinohana O, Sekerkova G, Santucci C, Marsala S, et al. Human neural stem cell replacement therapy for amyotrophic lateral sclerosis by spinal transplantation. *PLoS ONE*. Aug 20 2012;**7**(8):e42614

[73] Popescu IR, Nicaise C, Liu S, Bisch G, Knippenberg S, Daubie V, et al. Neural progenitors derived from human induced pluripotent stem cells survive and differentiate upon transplantation into a rat model of amyotrophic lateral sclerosis. *Stem Cells Translational Medicine*. 2013;**2**(3):167–174

- [74] Lee HJ, Kim KS, Ahn J, Bae HM, Lim I, Kim SU. Human motor neurons generated from neural stem cells delay clinical onset and prolong life in ALS mouse model. *PLoS ONE*. May 20 2014; **9**(5):e97518
- [75] Sun H, Hou Z, Yang H, Meng M, Li P, Zou Q, et al. Multiple systemic transplantations of human amniotic mesenchymal stem cells exert therapeutic effects in an ALS mouse model. *Cell and Tissue Research*. 2014; **357**(3):571-582
- [76] Kondo T, Funayama M, Tsukita K, Hotta A, Yasuda A, Nori S, et al. Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. *Stem Cell Reports*. 2014; **3**(2):242-249
- [77] Izrael M, Slutsky SG, Admoni T, Cohen L, Granit A, Hasson A, et al. Safety and efficacy of human embryonic stem cell-derived astrocytes following intrathecal transplantation in SOD1G93A and NSG animal models. *Stem Cell Research & Therapy*. 2018; **9**(1):152
- [78] Thomsen GM, Avalos P, Ma AA, Alkaslasi M, Cho N, Wyss L, et al. Transplantation of neural progenitor cells expressing glial cell line-derived neurotrophic factor into the motor cortex as a strategy to treat amyotrophic lateral sclerosis. *Stem Cells*. 2018; **36**(7): 1122-1131
- [79] Morrice JR, Gregory-Evans CY, Shaw CA. Animal models of amyotrophic lateral sclerosis: A comparison of model validity. *Neural Regeneration Research*. 2018; **13**:2050-4
- [80] Moura MC, Novaes MRCG, Zago YSSP, Eduardo EJ, Casulari LA. Efficacy of stem cell therapy in amyotrophic lateral sclerosis: A systematic review and meta-analysis. *Journal of Clinical Medical Research*. 2016; **8**(4):317-324
- [81] Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, Olivieri C, et al. Stem cell therapy in amyotrophic lateral sclerosis: A methodological approach in humans. *Amyotrophic Lateral Sclerosis: Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases*. 2003; **4**(3):158-161
- [82] Martinez HR, Gonzalez-Garza MT, Moreno-Cuevas JE, Caro E, Gutierrez-Jimenez E, Segura JJ. Stem-cell transplantation into the frontal motor cortex in amyotrophic lateral sclerosis patients. *Cytotherapy*. 2009; **11**(1):26-34
- [83] Prabhakar S, Marwaha N, Lal V, Sharma RR, Rajan R, Khandelwal N. Autologous bone marrow-derived stem cells in amyotrophic lateral sclerosis: A pilot study. *Neurology India*. 2012; **60**(5):465-469
- [84] Gamez J, Carmona F, Raguer N, Ferrer-Sancho J, Martín-Henao GA, Martí-Beltrán S, et al. Cellular transplants in amyotrophic lateral sclerosis patients: An observational study. *Cytotherapy*. 2010; **12**(5):669-677
- [85] Riley J, Federici T, Polak M, Kelly C, Glass J, Raore B, et al. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: A phase I safety trial, technical note, and lumbar safety outcomes. *Neurosurgery*. 2012; **71**(2): 405-416; discussion 416
- [86] Petrou P, Gothelf Y, Argov Z, Gotkine M, Levy YS, Kassir I, et al. Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factor transplantation in patients with amyotrophic lateral sclerosis: Results of phase 1/2 and 2a clinical trials. *JAMA Neurology*. 2016; **73**(3):337-344
- [87] Riley J, Glass J, Feldman EL, Polak M, Bordeau J, Federici T, et al. Intraspinal stem cell transplantation in

amyotrophic lateral sclerosis: A phase I trial, cervical microinjection, and final surgical safety outcomes. *Neurosurgery*. 2014;**74**(1):77-87

[88] Rushkevich YN, Kosmacheva SM, Zabrodets GV, Ignatenko SI, Goncharova NV, Severin IN, et al. The use of autologous mesenchymal stem cells for cell therapy of patients with amyotrophic lateral sclerosis in Belarus. *Bulletin of Experimental Biology and Medicine*. 2015;**159**(4):576-581

[89] Ruiz-López FJ, Blanquer M. Autologous bone marrow mononuclear cells as neuroprotective treatment of amyotrophic lateral sclerosis. *Neural Regeneration Research*. 2016;**11**(4): 568-569

[90] Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis: Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases*. 2000;**1**(5):293-299

[91] Yoon SH, Shim YS, Park YH, Chung JK, Nam JH, Kim MO, et al. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. *Stem Cells*. 2007; **25**(8):2066-2073

[92] Richman CM, Kinnealey A, Hoffman PC. Granulopoietic effects of lithium on human bone marrow in vitro. *Experimental Hematology*. 1981;**9**(4): 449-455

[93] Hashimoto R, Takei N, Shimazu K, Christ L, Lu B, Chuang D-M. Lithium induces brain-derived neurotrophic factor and activates TrkB in rodent cortical neurons: An essential step for

neuroprotection against glutamate excitotoxicity. *Neuropharmacology*. 2002;**43**(7):1173-1179

[94] Fornai F, Longone P, Cafaro L, Kastsichenka O, Ferrucci M, Manca ML, et al. Lithium delays progression of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;**105**(6):2052-2057

[95] Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, Khoo TK, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): A phase 3 multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Neurology*. 2013;**12**(4):339-345. DOI: 10.1016/S1474-4422(13)70037-1

[96] Verstraete E, Veldink JH, Huisman MHB, Draak T, Uijtendaal EV, van der Kooi AJ, et al. Lithium lacks effect on survival in amyotrophic lateral sclerosis: A phase IIb randomised sequential trial. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2012;**83**(5):557-564

[97] de Carvalho M, Scotto M, Lopes A, Swash M. Quantitating progression in ALS. *Neurology*. 2005;**64**(10):1783-1785

[98] Sane H, Sharma A, Gokulchandran N, Kalburgi S, Paranjape A, Badhe P. Neurorestoration in amyotrophic lateral sclerosis—A case report. *Journal of Stem cells and Regenerative Medicine*. 2016; **2**(1):29-37

[99] Sharma A, Sane H, Paranjape A, Sawant D, Inamdar S, Gokulchandran N, et al. Cellular therapy in motor neuron disease: A case report. *International Journal of Recent Advances in Multidisciplinary Research*. 2017;**4**(5):2605-2609