VOLUME - 10, ISSUE - 07, JULY- 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Neurology

ROLE OF REPRODUCTIVE HORMONAL STATUS ON THE OUTCOME OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS INTRATHECAL TRANSPLANTATION COMBINED WITH STANDARD TREATMENTS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS - A CONTROLLED COHORT STUDY

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ABSTRACT

OBJECTIVE: In this controlled follow-up study from March 2012 to June 2019 we investigated the effect of male and female reproductive hormones (MRH and FRH) on the outcomes of cellular transplantation in patients with probable and definite ALS (as per revised El-Escorial criteria), using Kaplan-Meier analysis; after intrathecal administration of autologous bone marrow derived mononuclear cells (BMMNCs), oral Lithium and standard rehabilitation.

METHODS: The intervention group was subdivided into pre-menopausal females (PRMF), post-menopausal females (POMF), pre-andropausal males (PRAM) (age < 40) and post andropausal males (POAM) (age > 40). Survival duration of the patients was compared between these subgroups and between intervention (n = 41) and control (n = 20) group.

RESULTS: Estimated survival in months was highest in PRMFs 88.5 ± 8.89, followed by POMFs 63.25 ± 9.2, PRAMs 61.25 ± 14.28 and POAMs 45.87 ± 3.59 (P = 0.040). Statistically significant survival-gain of 20.53 months (p=0.013) was seen in the intervention group (64.4 ± 4.95) compared to the control group (43.87 ± 6.68). No major adverse events were recorded.

CONCLUSION: Cellular therapy may improve the survival of the patients with ALS and outcome may be enhanced with better reproductive hormone (Estrogen, Progesterone and Testosterone) status due to the neuroprotective effect. FRH may give better results compared to MRH. Optimum levels of these hormones to achieve maximum benefit in the outcome needs to be investigated through larger cohort studies. Role of these hormones as potential treatment adjuvant should be assessed through interventional randomized controlled trials.

KEYWORDS : Bone marrow derived mononuclear cells (BMMNCs), Amyotrophic Lateral Sclerosis (ALS), cellular therapy, reproductive hormones, estrogen, progesterone, testosterone

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a debilitating neurodegenerative disorder that progresses with selective degeneration of anterior horn cells. Cumulative global incidence of ALS is 1.75 (1.55-1.96)/100,000 person-years of follow-up (1). This disorder has a heterogenous etiology and consequently, no definitive cure. It can only be managed via multidisciplinary intervention (2). Standard treatment involves Riluzole administration (3), which modestly extends survival in ALS patients; Edaravone, probably effective for early-stage treatment (4) and Nuedexta for symptomatic treatment of emotional lability (5).

Cellular therapy is being intensively investigated for treatment of ALS(6-16). Several pre-clinical (17-24) as well as clinical (6-16, 25-29) studies endorse the safety and efficacy of a variety of cells for ALS. Of these, autologous Bone marrow-derived Mononuclear Cells (BMMNCs) are safe (25,30) owing to their autologous nature and minimal manipulation outside of the host.

In our previously published study, we have shown that mean survival duration of patients who underwent intrathecal injection of autologous BMMNCs is higher compared to ALS controls; and, higher as compared to previous epidemiological studies (25). Patients with limb onset and oral Lithium administration showed better prognosis; and, age of symptom onset contributed significantly in improving patient survival. In the current controlled cohort study, we have investigated the effect of reproductive hormone status (premenopausal and preandropausal) on the outcome of cell therapy. We have also evaluated effect of cell therapy on the survival of patients with ALS using Kaplan-Meier (KM) analysis. We present the article in accordance with the TREND

reporting checklist.

MATERIALS AND METHODS

Study design and eligibility criteria for recruitment of patients

The primary aim is to investigate the effect of reproductive hormonal status (Estrogen, Progesterone and Testosterone) on the outcome of cell therapy. Secondary aim is to evaluate the effect of cell therapy on the survival of patients with ALS using Kaplan-Meier (KM) analysis.

Patients admitted to our institute from August 2013 to May 2017with the diagnosis of ALS were screened for the study. The International Statistical Classification of Diseases and Related Health Problems 10th Revision Version 2016 (31) was used for classifying the patients into ALS, Primary Lateral Sclerosis (PLS), Progressive Bulbar Palsy (PBP), Progressive Spinal Muscular Atrophy (PSMA) and Progressive Muscular Atrophy (PMA). Patients with ALS were selected, and further confirmed as having definite or probable ALS according to the revised El Escorial Criteria (32). Of these, the patients who underwent cellular therapy were included in the intervention group (n=41). For the control group, we selected patients diagnosed with definite or probable ALS from our out-patient department, who did not undergo cellular therapy (n=20). Findings from follow up at minimum 3 months duration till 87 months duration were included in this study. The study was a non-randomized controlled cohort study.

Sub-group analysis involved sectioning the intervention group according to type of symptom onset (limb vs. bulbar) and reproductive hormonal status (pre-andropause with symptom onset below 40 years of age vs. post-andropause with symptom onset at or above 40 years of age vs. pre-menopausal women at symptom onsets. post-menopausal women at symptom onset). Survival durations were compared across these groups.

INCLUSION CRITERIA

1. Clear diagnosis of definite or probable ALS as defined by the revised El Escorial Criteria (32)
2. Availability of an accurate symptom onset and survival timeline
3. Age of onset 30 to 70 years.

EXCLUSION CRITERIA

1. Patients diagnosed with other motor neuron disease or its mimics, such as PLS, PBP,PMA, progressive spinal muscular atrophy, adult onset spinal muscular atrophy, Hirayama disease, monomelic amyotrophy, and madras motor neuron disease;
2. Patients who received intramuscular cell injections;
3. Patients who received Edaravone injections (As the drug was approved by FDA in the middle of the study duration, some patients had received the drug while others did not; this could have confounded the survival analysis hence these patients were excluded).
4. Patients with co-morbidities such as presence of acute infections (HIV/HBV/HCV), malignancies, bleeding tendencies, renal and hepatic dysfunction, and other acute medical conditions such as respiratory infection and pyrexia.

INTERVENTION

Provision of intervention was based on the Revised World Medical Association Helsinki Declaration for Ethical Principles for Medical Research Involving Human Subjects (33), with ethical approval from the Institutional Ethics Committee. An informed consent was obtained from every patient. All the patients underwent a thorough clinical evaluation to ascertain pre-intervention surgical fitness. 48 hours and 24 hours before aspiration of bone marrow, all patients were subcutaneously administered Granulocyte-Colony Stimulating Factor (G-CSF). G-CSF has been shown to enhance the rate of BMMNC proliferation within the bone marrow (34), leading to a higher yield.

Cell transplantation was carried out as described in our previously published study (25). Briefly, 100 ml to 120 ml of bone marrow was aspirated under local anesthesia from the anterior superior iliac spine and transferred aseptically to the regenerative medicine laboratory. Density gradient centrifugation separated the mononuclear cell fraction, which was washed and analysed for cell viability manually under a microscope using Trypan blue exclusion, as well as using a TALI counter for confirmation. On an average, 1.078×10^8 cells were obtained with an average viability of 96.83%. Individual samples were also subjected to CD34+ characterization using Fluorescence Activated Cell Sorting with the CD34 PE antibody.

Separated autologous BMMNCs were reintroduced into the patient the same day after dilution in patient's own CSF, as it provides a conducive environment to the cells for efficient migration and grafting (35). This procedure was carried out in an operation theatre under aseptic conditions. Intrathecal administration was performed by a qualified neurosurgeon using a lumbar puncture at L4-L5 level. Intravenous methyl prednisolone (20 mg/kg) in 500 ml Ringer's Lactate was simultaneously administered to assuage local inflammation. The entire procedure including the bone marrow aspiration, cell isolation and cell transplantation was completed on the same day, within 4 – 5 hours.

Following the procedure, patients were closely monitored for any immediate adverse events. They were administered a structured, multidisciplinary neurorehabilitatory regime and advised to continue the same post discharge, along with standard Riluzole treatment. Additionally, patients were prescribed 300 mg Lithium once daily or twice daily. Serum Lithium levels were checked on third day to prevent reaching toxicity levels and maintained within the therapeutic range of 0.4 -0.8 mmol/L. All patients were followed up either telephonically or physically thereafter every 3 months.

Adverse Events monitoring

Post procedure, all the patients were closely monitored during the hospital stay to capture any adverse events. Adverse events were documented in two different categories, procedure related (Spinal headache, pain and redness at the site of injection or aspiration, nausea, vomiting and swelling at the aspiration or injection site) and cellular transplantation related adverse events (Neurological deterioration).

STATISTICAL ANALYSIS

Baseline Statistics:

Baseline demographic data of all the patients was gathered. Mean age at symptom onset was computed across intervention and control groups. Descriptive statistics for the demographic data was calculated as mean (standard deviation).

Survival and Progression Analysis:

Survival was assessed using KM survival analysis and compared across controls and intervention using log rank test and calculated as mean (standard error).

Subgroup Analysis:

Within the intervention group, subgroup analysis was performed by first confirming equal distribution of baseline demographics across the comparison subgroups. Survival duration was compared between the subgroups across various axes such as type of symptom onset (limb vs. bulbar), and reproductive hormone status (Pre-andropausal vs. Post-andropausal vs. Pre-menopausal vs. post-menopausal)

Statistical analysis was performed using IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

RESULTS

Baseline Statistics:

There were 41 patients in the intervention group and 20 patients in the control group who completed the study follow up duration. Baseline statistics showed consistency across the control and intervention groups. The mean age at symptom onset for the control group was 54 (8.68) years, and for the intervention group was 49 (10.48) years.

Survival and Progression Analysis:

Survival analysis of the control group versus intervention showed that on an average, the intervention group survived for 64.4 (4.95) months, while the control group survived for 43.87(6.68) months; the difference of 20.53 months was statistically significant with a p-value of 0.013 (Figure 1). Percentage analysis for the two cohorts, shows a higher survival percentage of 46.3% in intervention group as compared to 30% in the control group.

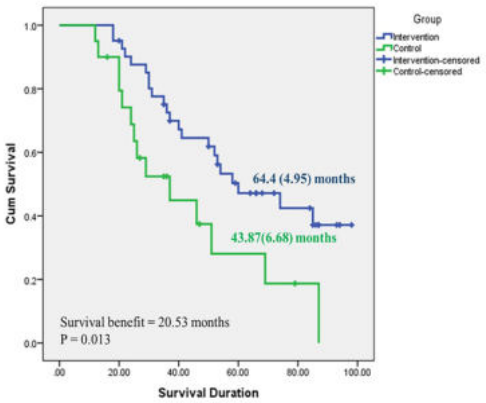

Figure 1: Kaplan-Meier graph showing comparison of survival duration between intervention and control group

Subgroup Analysis:

We subdivided data collected for the intervention group across several axes and analysed the subgroups to observe intra-group differences. The following parameters were assessed:

I. Type of symptom onset: Limb vs. Bulbar;

ii. Pre-andropausal males vs. Post-andropausal males vs. Pre-menopausal females vs. Post-menopausal females

Type of onset: Limb vs. Bulbar

Within the intervention cohort, those with limb onset of symptoms (n=32) survived for 69.78 (5.49) months, while those with bulbar symptom onset (n=9) survived for 39.04 (4.48) months; there was a statistically significant survival difference of 30.74 months (p=0.012) (Figure 2).

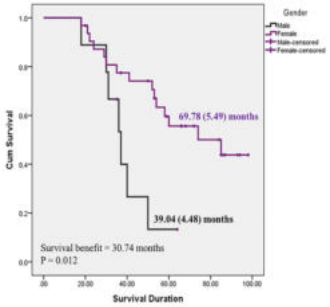

Figure 2: Kaplan-Meier graph showing comparison of survival duration between patients with limb onset and bulbar onset

Reproductive hormonal status:

We compared the estimated survival duration of four groups: pre-menopausal women, post-menopausal women, pre-andropausal men and post-andropausal men. We found that pre-menopausal women show highest survival, with an average of 88.50 (8.88) months, followed by post-menopausal women, with an average of 63.25 (9.21) months, followed by pre-andropausal males, with an average of 61.25 (14.28) months, and lastly, post-andropausal men, showed the lowest survival of 45.87 (3.59) months. There was a statistically significant difference between the survival of these patients (P= 0.040) (Figure 3).

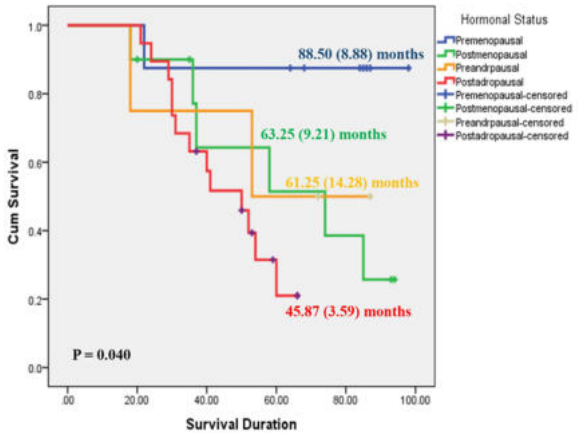

Figure 3: Kaplan-Meier graph showing comparison of survival duration of patients based on hormonal status

Adverse Events monitoring:

Post procedure, few patients experienced procedure related minor adverse events like nausea, vomiting and spinal headache but there were no long-term adverse events. There were no adverse events related to cellular transplantation. One patient had to discontinue Lithium as he developed adverse reactions to Lithium.

DISCUSSION

A heterogenous pathophysiological landscape is the biggest hurdle encountered by clinicians worldwide for the treatment of ALS. Various mechanisms have been presumed to contribute to this neuropathophysiology, including but not limited to involvement of misfolded ubiquitinated aggregates (36-38), excessive free radicals (39-41), excitotoxicity (42), mitochondrial abnormality (43), widespread neuro inflammation (44), axonal transport dysfunction (45) and synaptic failure (46).

BMMNCs confer neuroprotection through paracrine effects by releasing and regulating neurotrophic factors, like brain-derived neurotrophic factor (BDNF) (47), ciliary neurotrophic factor (CNTF) and glial cell–derived neurotrophic factor (GDNF) (48), □-nerve growth factor (□-NGF) (49), vascular endothelial growth factor (VEGF) (50), tumor necrosis factor (TNF)-□ (51), basic fibroblast growth factor (bFGF) (50), platelet-derived growth factor-BB (PDGF-BB) (49), connective tissue growth factor (CTGF) and fibroblast growth factors (FGF) 2 and 7 (48), hepatic growth factor (HGF) and Insulin growth factor (IGF)-1 (52), and angiopoietin 1 (ANG-1) (53), as well as interleukins (IL-1α, IL-β, IL-6, IL-10) (51).

Further, these cells have also been shown to migrate, multiply and differentiate into myelinating, glial and neuronal phenotypes (54-56);integrating into and repairing damaged tissue. Secretion of VEGF, FGF, and bFGF triggers a cascade of events that culminates in neoangiogenesis and improved blood circulation (50). Reduced levels of TNF-α and increased levels of IL-10 lead to an anti-inflammatory effect on the neural microenvironment (48,53,57). Innate and adaptive response is also regulated by cells via secretion of Tumor growth factor

(TGF)-β and elevation of regulatory T cells (Tregs) and T helper-2 cells (Th2 cells) (58). Soluble factors from cells also significantly upregulate the expression of glutamate transporters in ALS astrocytes, enhancing glutamate clearance from the synapse (52). Adult stem cells have been demonstrated as safe and efficacious as a therapy for ALS by multiple studies (9,25,59,60). None of the studies have shown any major or irreversible adverse events post transplantation.

Neuroprotective effects of reproductive hormones:

In pre-clinical studies, progesterone has been shown to reduce reactive gliosis due to anti-inflammatory effects preventing neurodegeneration (61). In a preliminary study, endogenous progesterone levels were found to be negatively correlated with age and positively corelated with survival. Limb onset, slow progression and diagnostic delay showed a trend towards positive correlation (62) suggesting a neuroprotective role of progesterone in ALS.

Multiple groups report evidence that the females have an advantage in being protected from the pathology of ALS is due to the protective role of Estrogen(E2) (63,64). In an ALS mouse model carrying the human Cu/Zn superoxide dismutase (hSOD1) G93A transgene, E2 treatment improved the lifespans in overiectomized females, and high-dose E2 treatment significantly delayed disease progression of ovariectomized hSOD1G93A transgenic mice. E2 has also been shown to exert profound protective effects against stroke-like ischemic injury in female rats (65) mediated by estrogen receptors (66), suggesting a global neuroprotective role of E2. Klemann et al (67) found that E2 signaling is functionally involved in inter-linked processes crucial to maintain axonal functionality, especially of the long axons of motor neurons, and that E2 may be a protective factor in ALS, especially for bulbar-onset. Heitzer et al (68) show that symptomatic mice with E2 substitution exhibit improved motor performance, along with significantly reduced expression of inflammasome proteins, activated caspase 1 (initiator of apoptosis) levels and mature interleukin-1β, correlating with an increased survival of motor neurons in the lumbar spinal cord. Further, Johann et al. (69) indicate that E2 can influence the cholinergic system by increasing choline acetyltransferase (ChAT) expression in the mouse spinal cord, which is typically reduced in ALS (70). Singer et al (71) provide evidence that activation of the MAPK pathway by estrogen participates in mediating neuroprotection via an estrogen receptor. Nakamizo et al. (72) show that E2 and its biologically inactive stereoisomer, 17α-estradiol, prevented glutamate- and nitric oxide (NO)-induced selective motor neuronal death observed in primary cultures of the rat spinal cord. These pre-clinical studies indicate that E2 may have application as a treatment for ALS.

Testosterone also provides neuroprotection. It is important in the development of nervous system. Reproductive hormones are also responsible for the sexual dimorphism in the neuronal development. Decrease in the testosterone levels reduces the dendritic length (73).Free serum testosterone has been known to be reduced overall in ALS patients in one study (74). Another study showed that testosterone remained stable in ALS patients compared to control subjects. However, subgroup analysis revealed that patients with higher testosterone levels and lower progesterone/free testosterone ratio had a worsened monthly forced vital capacity (75). In our previously published study, we noted that there was a statistically significant positive co-relation between serum testosterone levels and ALS-FRSr scale. This suggests that as the disease progressed testosterone levels also declined and highlights that absence of testosterone may accelerate the neurological damage (76).

Few studies have investigated the link between female reproductive hormones and ALS. The most relevant findings come from a population-based, case–controlled study in the Netherlands by de Jong et al (77) including 131 female ALS patients and 430 female controls. Their results demonstrate that increasing the reproductive time-span by a year decreases the risk of ALS with an odds ratio of 0.95 (p=0.005). Each year longer reproductive time-span and lifetime endogenous estrogen exposure were associated with a longer survival of ALS patients. This may imply that female hormones serve neuroprotective effects in ALS pathology. However, Rudnicki et al. (78) raise the question as to whether estrogen may be neuroprotective in delaying or preventing ALS. From their findings, there was no difference in survival in those patients taking estrogen compared to those not on the medication. Popat et al. (79) found in their case-controlled study that reproductive factors such as age at menarche, age at final menstrual period, parity, oral contraceptive use, and type of menopause (natural vs. hysterectomy with or without oophorectomy) were not associated with risk of ALS. From their study, postmenopausal hormone replacement, due to lower levels of estrogen and progesterone, was positively, but not significantly, associated with the risk of ALS. Nevertheless, the studies by Rudnicki et al. (78) and Popat et al. (79) differ from study by de Jong et al. (77), as they only investigate the effects of hormone replacement therapy for ALS afflicted females. These studies do not interrogate whether onset of symptoms for these women was pre-menopausal or post-menopausal. Our results suggest that higher levels of female reproductive hormones improve survival in patients, suggesting augmentation of neuroprotective benefits of cellular therapy by these hormones.

Our results suggest that reproductive hormones may resonate with the neuroprotective properties of BMMNCs. In both males and females, outcome after cellular therapy was found to be better in the groups with higher levels of reproductive hormones. Pre-andropausal males survived significantly longer than post-andropausal males. Pre-menopausal women showed a higher survival duration as compared to post-menopausal women. Premenopausal women had higher survival as compared to pre-andropausal males. Collectively, this data hints at the plausibility of a predominant neuroprotective effect of female hormones in ALS contributing to enhanced outcome after cellular therapy.

Conclusion and Future Directions

Various studies suggest that autologous bone marrow mononuclear cells are safe and effective in improving the survival and mitigating disease progression in patients with ALS; all that remains to be done is fine-tuning this therapy to obtain optimum results. This response is dependent on various factors, like age of symptom onset, type of symptom onset (limb vs. bulbar) and gender. In addition to these factors, the current study points out the role of reproductive hormonal status and its effect in augmenting the outcome of cellular therapy. Multitude of preclinical as well as clinical studies endorse the neuroprotective action of reproductive hormones in ALS. Investigating the link between responsiveness to cell therapy, reproductive hormones and ALS promises to unlock new therapeutic avenues for ALS treatment. Findings of our study suggest that survival benefit achieved with cellular therapy can be enhanced in presence of these hormones. Furthermore, female reproductive hormones (Estrogen and Progesterone) may give better results as compared to male reproductive hormones (Testosterone). Optimum levels of these hormones to achieve maximum benefit in the outcome needs to be investigated through larger cohort studies. Role of these hormones as potential treatment adjuvant should be assessed through interventional randomized controlled trials.

Conflict of Interest

The authors declare no conflicts of interest.

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Manuscript writing: All authors
Final approval of manuscript: All authors
Footnote:
The authors have completed the TREND reporting checklist
The authors declare there is no conflict of interest
The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Marin B, Boumédiene F, Logroscino G, Couratier P, Babron MC, Leutenegger AL, Copetti M, Preux PM, Beghi E. Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. International journal of epidemiology. 2017 Feb 1;46(1):57-74.
2. Martin S, Trevor-Jones E, Khan S, Shaw K, Marchment D, Kulka A, Ellis CE, Burman R, Turner MR, Carroll L, Mursaleen L. The benefit of evolving multidisciplinary care in ALS: a diagnostic cohort survival comparison. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2017 Oct 2;18(7-8):569-75.
3. Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Amyotrophic lateral sclerosis and other motor neuron disorders: official publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases. 2003 Sep;4(3):191-206.
4. Abe K, Aoki M, Tsuji S, Itoyama Y, Sobue G, Togo M, Hamada C, Tanaka M, Akimoto M, Nakamura K, Takahashi F. Safety and efficacy of edaravone in well-defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. The Lancet Neurology. 2017 Jul 1;16(7):505-12.
5. Smith R, Pioro E, Myers K, Sirdofsky M, Goslin K, Meekins G, Yu H, Wymer J, Cudkowicz M, Macklin EA, Schoenfeld D. Enhanced bulbar function in amyotrophic lateral sclerosis: the Nuedexta treatment trial. Neurotherapeutics. 2017 Jul 1;14(3):762-72.
6. Ciervo Y, Ning K, Jun X, Shaw PJ, Mead RJ. Advances, challenges and future directions for stem cell therapy in amyotrophic lateral sclerosis. Molecular neurodegeneration. 2017 Dec;12(1):85.
7. Huang H, Chen L, Xi H, Wang H, Zhang J, Zhang F, Liu Y. Fetal olfactory ensheathing cells transplantation in amyotrophic lateral sclerosis patients: a controlled pilot study. Clinical transplantation. 2008 Nov;22(6):710-8.
8. Glass JD, Boulis NM, Johe K, Rutkove SB, Federici T, Polak M, Kelly C, Feldman EL. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: results of a phase I trial in 12 patients. Stem cells. 2012 Jun;30(6):1144-51.
9. Blanquer M, Moraleda JM, Iniesta F, Gómez-Espuch J, Meca-Lallana J, Villaverde R, Pérez-Espejo MÁ, Ruíz-López FJ, Garcia Santos JM, Bleda P, Izura V. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: a pilot safety study. Stem cells. 2012 Jun;30(6):1277-85.
10. Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S, Carriero A, Monaco F, Fagioli F. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. Cytotherapy. 2012 Jan 1;14(1):56-60.
11. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, Bulte JW, Petrou P, Ben-Hur T, Abramsky O, Slavin S. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Archives of neurology. 2010 Oct 11;67(10):1187-94.
12. Petrou P, Gothelf Y, Argov Z, Gotkine M, Levy YS, Kassis I, Vaknin-Dembinsky A, Ben-Hur T, Offen D, Abramsky O, Melamed E. 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 Mar 1;73(3):337-44.
13. Oh KW, Moon C, Kim HY, Oh SI, Park J, Lee JH, Chang IY, Kim KS, Kim SH. Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem cells translational medicine. 2015 Jun;4(6):590-7.
14. Syková E, Rychmach P, Drahorádová I, Konrádová Š, Růžičková K, Voříšek I, Forostyak S, Homola A, Bojar M. Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: results of phase I/IIa clinical trial. Cell transplantation. 2017 Apr;26(4):647-58.
15. 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 Sep 1;60(5):465.
16. Ferrero I, Mazzini L, Rustichelli D, Gunetti M, Mareschi K, Testa L, Nasuelli N, Oggioni GD, Fagioli F. Bone marrow mesenchymal stem cells from healthy donors and sporadic amyotrophic lateral sclerosis patients. Cell transplantation. 2008 Mar;17(3):255-66.
17. Xu L, Yan J, Chen D, Welsh AM, Hazel T, Johe K, Hatfield G, Koliatsos VE. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. Transplantation. 2006 Oct 15;82(7):865-75.V
18. Popescu IR, Nicaise C, Liu S, Bisch G, Knippenberg S, Daubie V, Bohl D, Pochet R. 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 Mar;2(3):167-74.
19. Hefferan MP, Galik J, Kakinohana O, Sekerkova G, Santucci C, Marsala S, Navarro R, Hruska-Plochan M, Johe K, Feldman E, Cleveland DW. Human neural stem cell replacement therapy for amyotrophic lateral sclerosis by spinal transplantation. PloS one. 2012;7(8).
20. Garbuzova-Davis S, Rodrigues MC, Mirtyl S, Turner S, Mitha S, Sodhi J, Suthakaran S, Eve DJ, Sanberg CD, Kuzmin-Nichols N, Sanberg PR. Multiple intravenous administrations of human umbilical cord blood cells benefit in a mouse model of ALS. PLoS One. 2012;7(2).
21. Lee JC, Seong J, Kim SH, Lee SJ, Cho YJ, An J, Nam DH, Joo KM, Cha CI. Replacement of microglial cells using Clodronate liposome and bone marrow transplantation in the central nervous system of SOD1G93A transgenic mice as an in vivo model of amyotrophic lateral sclerosis. Biochemical and biophysical research communications. 2012 Feb 10;418(2):359-65.
22. Kondo T, Funayama M, Tsukita K, Hotta A, Yasuda A, Nori S, Kaneko S, Nakamura M, Takahashi R, Okano H, Yamanaka S. Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. Stem cell reports. 2014 Aug 12;3(2):242-9.
23. Uccelli A, Milanese M, Principato MC, Morando S, Bonifacino T, Vergani L, Giunti D, Voci A, Carminati E, Giribaldi F, Caponnetto C. Intravenous mesenchymal stem cells improve survival and motor function in experimental amyotrophic lateral sclerosis. Molecular Medicine. 2012 May;18(5):794-804.
24. Sun H, Hou Z, Yang H, Meng M, Li P, Zou Q, Yang L, Chen Y, Chai H, Zhong H, Yang ZZ. Multiple systemic transplantations of human amniotic mesenchymal stem cells exert therapeutic effects in an ALS mouse model. Cell and tissue research. 2014 Sep 1;357(3):571-82.
25. Sharma AK, Sane HM, Paranjape AA, Gokulchandran N, Nagrajan A, D'sa M, Badhe PB. 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.
26. Tang BL. The use of mesenchymal stem cells (MSCs) for amyotrophic lateral sclerosis (ALS) therapy–a perspective on cell biological mechanisms. Reviews in the Neurosciences. 2017 Oct 26;28(7):725-38.
27. Riley J, Federici T, Polak M, Kelly C, Glass J, Raore B, Taub J, Kesner V, Feldman EL, Boulis NM. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I safety trial, technical note, and lumbar safety outcomes. Neurosurgery. 2012 Aug 1;71(2):405-16.
28. Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, Olivieri C, Pastore I, Marasso R, Madon E. Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders. 2003 Sep 1;4(3):158-61.
29. Martínez HR, Molina-Lopez JF, González-Garza MT, Moreno-Cuevas JE, Caro-Osorio E, Gil-Valadez A, Gutierrez-Jimenez E, Zazueta-Fierro OE, Meza JA, Couret-Alcaraz P, Hernandez-Torre M. Stem cell transplantation in amyotrophic lateral sclerosis patients: methodological approach, safety, and feasibility. Cell transplantation. 2012 Sep;21(9):1899-907.
30. Ruiz-López FJ, Blanquer M. Autologous bone marrow mononuclear cells as neuroprotective treatment of amyotrophic lateral sclerosis. Neural regeneration research. 2016 Apr;11(4):568.
31. World Health Organization. ICD-10 Version: 2016. 2016. International Statistical Classification of Diseases and Related Health Problems 10th Revision URL: https://icd. who. int/browse10/2016/en [accessed 2018-05-08][WebCite Cache ID 6zGP3Tx2y]. 2015.
32. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis and other motor neuron disorders. 2000 Jan 1;1(5):293-9.
33. Carlson RV, Boyd KM, Webb DJ. The revision of the Declaration of Helsinki: past, present and future. British journal of clinical pharmacology. 2004 Jun;57(6):695-713.
34. Zhang XM, Du F, Yang D, Wang R, Yu CJ, Huang XN, Hu HY, Liu W, Fu J. Granulocyte colony-stimulating factor increases the therapeutic efficacy of bone marrow mononuclear cell transplantation in cerebral ischemia in mice. BMC neuroscience. 2011 Dec 1;12(1):61.
35. Ren C, Yin P, Ren N, Wang Z, Wang J, Zhang C, Ge W, Geng D, Wang X. Cerebrospinal fluid-stem cell interactions may pave the path for cell-based therapy in neurological diseases. Stem cell research & therapy. 2018 Dec;9(1):66.
36. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006 Oct 6;314(5796):130-3.
37. Nishimura AL, Župunski V, Troakes C, Kathe C, Fratta P, Howell M, Gallo JM, Hortobágyi T, Shaw CE, Rogelj B. Nuclear import impairment causes cytoplasmic trans-activation response DNA-binding protein accumulation and is associated with frontotemporal lobar degeneration. Brain. 2010 Jun 1;133(6):1763-71.
38. Arnold ES, Ling SC, Huelga SC, Lagier-Tourenne C, Polymenidou M, Ditsworth D, Kordasiewicz HB, McAlonis-Downes M, Platoshyn O, Parone PA, Da Cruz S. ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. Proceedings of the National Academy of Sciences. 2013 Feb 19;110(8):E736-45.
39. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, Rahmani Z. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993 Mar;362(6415):59-62.
40. Borchelt DR, Lee MK, Slunt HS, Guarnieri M, Xu ZS, Wong PC, Brown RH, Price DL, Sisodia SS, Cleveland DW. Superoxide dismutase 1 with mutations linked to familial amyotrophic lateral sclerosis possesses significant activity. Proceedings of the National Academy of Sciences. 1994 Aug 16;91(17):8292-6.
41. Catania MV, Aronica E, Yankaya B, Troost D. Increased expression of neuronal nitric oxide synthase spliced variants in reactive astrocytes of amyotrophic lateral sclerosis human spinal cord. Journal of Neuroscience. 2001 Jun 1;21(11):RC148-.
42. Battaglia G, Bruno V. Metabotropic glutamate receptor involvement in the

pathophysiology of amyotrophic lateral sclerosis: new potential drug targets for therapeutic applications. Current opinion in pharmacology. 2018 Feb 1;38:65-71.
43. Smith EF, Shaw PJ, De Vos KJ. The role of mitochondria in amyotrophic lateral sclerosis. Neuroscience letters. 2019 Sep 25;710:132933.
44. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010 Mar 19;140(6):918-34.
45. Korac J, Schaeffer V, Kovacevic I, Clement AM, Jungblut B, Behl C, Terzic J, Dikic I. Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. Journal of cell science. 2013 Jan 15;126(2):580-92.
46. Martin LJ, Chang Q. Inhibitory synaptic regulation of motoneurons: a new target of disease mechanisms in amyotrophic lateral sclerosis. Molecular neurobiology. 2012 Feb 1;45(1):30-42.
47. Phruksaniyom C, Dharmasaroja P, Issaragrisil S. Bone marrow non-mesenchymal mononuclear cells induce functional differentiation of neuroblastoma cells. Experimental hematology & oncology. 2013 Dec;2(1):9.
48. Pastor D, Viso-León MC, Jones J, Jaramillo-Merchán J, Toledo-Aral JJ, Moraleda JM, Martínez S. Comparative effects between bone marrow and mesenchymal stem cell transplantation in GDNF expression and motor function recovery in a motorneuron degenerative mouse model. Stem cell reviews and reports. 2012 Jun 1;8(2):445-58.
49. Moniche F, Montaner J, Gonzalez-Marcos JR, Carmona M, Piñero P, Espigado I, Cayuela A, Escudero I, De La Torre-Laviana FJ, Boada C, Rosell A. Intra-arterial bone marrow mononuclear cell transplantation correlates with GM-CSF, PDGF-BB, and MMP-2 serum levels in stroke patients: results from a clinical trial. Cell transplantation. 2014 Jan;23(1_suppl):57-64.
50. Bhasin A, Srivastava MP, Mohanty S, Vivekanandhan S, Sharma S, Kumaran S, Bhatia R. Paracrine mechanisms of intravenous bone marrow-derived mononuclear stem cells in chronic ischemic stroke. Cerebrovascular diseases extra. 2016;6(3):107-19.
51. Brenneman M, Sharma S, Harting M, Strong R, Cox Jr CS, Aronowski J, Grotta JC, Savitz SI. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. Journal of Cerebral Blood Flow & Metabolism. 2010 Jan;30(1):140-9.
52. Gu R, Hou X, Pang R, Li L, Chen F, Geng J, Xu Y, Zhang C. Human adipose-derived stem cells enhance the glutamate uptake function of GLT1 in SOD1G93A-bearing astrocytes. Biochemical and biophysical research communications. 2010 Mar 12;393(3):481-6.
53. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A. 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 Aug 28;104(9):1046-52.
54. Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis JD. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. Glia. 2001 Jul;35(1):26-34.
55. 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 Dec 1;290(5497):1779-82.
56. 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. 1997 Apr 15;94(8):4080-5.
57. Schneider A, Krüger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gassler N, Mier W, Hasselblatt M. The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. The Journal of clinical investigation. 2005 Aug 1;115(8):2083-98.
58. Kim SH, Oh KW, Jin HK, Bae JS. Immune inflammatory modulation as a potential therapeutic strategy of stem cell therapy for ALS and neurodegenerative diseases. BMB reports. 2018 Nov;51(11):545.
59. Syková E, Homola A, Mazanec R, Lachmann H, Konrádová ŠL, Kobylka P, Pádr R, Neuwirth J, Komrska V, Vávra V, Štulík J. Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell transplantation. 2006 Sep;15(8-9):675-87.
60. Sharma A, Sane H, Kalburgi S, Paranjape A, Gokulchandran N, Badhe P. Potential Benefits of Cellular Transplantation in a Patient with Amyotrophic Lateral Sclerosis. Current Opinions in Neurological Science. 2017;1(1):31-43.
61. Gargiulo Monachelli G, Meyer M, Rodriguez GE, Garay LI, Sica RE, De Nicola AF, Gonzalez Deniselle MC. Endogenous progesterone is associated to amyotrophic lateral sclerosis prognostic factors. Acta neurologica scandinavica. 2011 Jan;123(1):60-7.
62. Heitzer M, Kaiser S, Kanagaratnam M, Zendedel A, Hartmann P, Beyer C, Johann S. Administration of 17□-estradiol improves motoneuron survival and down-regulates inflammasome activation in male SOD1 (G93A) ALS mice. Molecular neurobiology. 2017 Dec 1;54(10):8429-43.
63. Choi CI, Lee YD, Gwag BJ, Cho SI, Kim SS, Suh-Kim H. Effects of estrogen on lifespan and motor functions in female hSOD1 G93A transgenic mice. Journal of the neurological sciences. 2008 May 15;268(1-2):40-7.
64. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. Journal of Cerebral Blood Flow & Metabolism. 1998 Nov;18(11):1253-8.
65. Wise PM, Dubal DB, Wilson ME, Rau SW, Böttner M, Rosewell KL. Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Research Reviews. 2001 Nov 1;37(1-3):313-9.
66. Militello A, Vitello G, Lunetta C, Toscano A, Maiorana G, Piccoli T, La Bella V. The serum level of free testosterone is reduced in amyotrophic lateral sclerosis. Journal of the neurological sciences. 2002 Mar 15;195(1):67-70.
67. Klemann CJ, Visser JE, Van Den Bosch L, Martens GJ, Poelmans G. Integrated molecular landscape of amyotrophic lateral sclerosis provides insights into disease etiology. Brain Pathology. 2018 Mar;28(2):203-11.
68. Heitzer M, Kaiser S, Kanagaratnam M, Zendedel A, Hartmann P, Beyer C, Johann S. Administration of 17β-estradiol improves motoneuron survival and down-regulates inflammasome activation in male SOD1 (G93A) ALS mice. Molecular neurobiology. 2017 Dec 1;54(10):8429-43.
69. Johann S, Dahm M, Kipp M, Zahn U, Beyer C. Regulation of Choline Acetyltransferase Expression by 17β-Oestradiol in NSC-34 Cells and in the Spinal Cord. Journal of neuroendocrinology. 2011 Sep;23(9):839-48.
70. Crochemore C, Peña-Altamira E, Virgili M, Monti B, Contestabile A. Disease-related regressive alterations of forebrain cholinergic system in SOD1 mutant transgenic mice. Neurochemistry international. 2005 Apr 1;46(5):357-68.
71. Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. Journal of Neuroscience. 1999 Apr 1;19(7):2455-63.
72. Nakamizo T, Urushitani M, Inoue R, Shinohara A, Sawada H, Honda K, Kihara T, Akaike A, Shimohama S. Protection of cultured spinal motor neurons by estradiol. Neuroreport. 2000 Nov 9;11(16):3493-7.
73. Bia³ek M, Zaremba P, Borowicz KK, Czuczwar SJ. Neuroprotective role of testosterone in the nervous system. Pol. J. Pharmacol. 2004;56:509-18.
74. Militello A, Vitello G, Lunetta C, Toscano A, Maiorana G, Piccoli T, La Bella V. The serum level of free testosterone is reduced in amyotrophic lateral sclerosis. Journal of the neurological sciences. 2002 Mar 15;195(1):67-70.
75. Gargiulo-Monachelli GM, Sivori M, Meyer M, Sica RE, De Nicola AF, Gonzalez-Deniselle MC. Circulating gonadal and adrenal steroids in amyotrophic lateral sclerosis: possible markers of susceptibility and outcome. Hormone and Metabolic Research. 2014 Jun;46(06):433-9.
76. Sane H, Varghese R, Paranjape A, Jain R, Gokulchandran N, Badhe P, Sharma A. Correlation of Testosterone levels with progression of Amyotrophic Lateral Sclerosis in Males: A Cross-Sectional Study. Age (years). 2019 Jun 15;48(10.2):25.
77. de Jong S, Huisman M, Sutedja N, van der Kooi A, de Visser M, Schelhaas J, van der Schouw Y, Veldink J, van den Berg L. Endogenous female reproductive hormones and the risk of amyotrophic lateral sclerosis. Journal of neurology. 2013 Feb 1;260(2):507-12.
78. Rudnicki SA. Estrogen replacement therapy in women with amyotrophic lateral sclerosis. Journal of the neurological sciences. 1999 Oct 31;169(1-2):126-7.
79. Popat RA, Van Den Eeden SK, Tanner CM, Bernstein AL, Bloch DA, Leimpeter A, McGuire V, Nelson LM. Effect of reproductive factors and postmenopausal hormone use on the risk of amyotrophic lateral sclerosis. Neuroepidemiology. 2006;27(3):117-21.