The First in Human Case of Amyotrophic Lateral Sclerosis Treated with Stem Cell-Derived Conditioned Medium: A 1-Year- Follow Up

Minoru Ueda[1,2]* and Yasuhiro Seta[3]

[1]*Department of Medicine, Postgraduate School of Medicine, Emeritus of Nagoya University, Nagoya, Japan*

[2]*President of SAISEIKEN Co. LTD, Tokyo, Japan*

[3]*Director of Smart Clinic Tokyo, Tokyo, Japan*

Corresponding Author*

Minoru Ueda
Department of Medicine
Postgraduate School of Medicine,
Emeritus of Nagoya University, Nagoya, Japan
President of SAISEIKEN Co. LTD, Tokyo, Japan
E-mail: info@saisei-ken.com

Received date: 25 April 2022; Manuscript No. NNR-22-61741; **Editor assigned:** 26-April-2022; Pre QC No. NNR-22-61741 (PQ); **Reviewed:** 29-April-2022; QC No. NNR-22-61741 (Q); **Revised Date:** 5-May-2022, Manuscript No. NNR-22-61741 (R); **Published:** 16-May-2022; DOI. 10.37532/nnr.22.4.1.1-11

Abstract

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a devasting neuroinflammatory disease of the Central Nerve System (CNS) and current ALS treatments do not result in complete remission leading to death 3-5 years in most cases. Here we report the intriguing case of an ALS patient treated by transnasal and intravenous administration of stem cell-conditioned medium and clinical improvement in disease severity and delay of disease progression.

Case Report: In a 68-years-old male patient suffering from muscle weakness and fasciculations, progressive muscular atrophy, a variant of ALS, was diagnosed after extensive examinations ruling out other diseases in April 2019 in Yokohama city hospital and followed by Kitasato university hospital from June 2020, then finally referred to our clinic in January 2021. In our clinic, the patient received transnasal and intravenous administration of the Stem Cell From Human Exfoliated Deciduous Teeth Derived Conditioned Medium (SHEDCM) from September 2021. The success of the therapy was followed by ALS Functional Rating Score-Revised (ALSFRS-R) and Range Of Motion (ROM). Pulmonary function was monitored by SpO2 continuously in his home and by pulmonary function test in the university hospital. Unexpected improvements occurred one week after starting intravenous therapy with SHEDCM, ROM in limbs and neck expanded remarkably and continued this amelioration for the following 5 months. This ameliorated symptom in ROM confirmed the remission of one of the typical signs of ALS, spasticity contracture. It improved the patient's quality of life and activities of daily living. During the period in this therapy, we did not experience any adverse effects related to thistherapy. Conclusion: The therapy described here may be a promising approach to treating some kinds of motor neuron disease such as ALS.

Keywords: Amyotrophic lateral sclerosis • Dental pulp stem cell • Conditioned medium • Spastic contracture • Rang of motion • The first in human

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a disease of unknown etiology in which the muscles of the limbs, throat, tongue and respiratory system gradually deteriorate and lose strength, and has been designated an intractable disease by the International Government. In ALS, the problem does not originate with the muscles themselves, but rather with degeneration of the upper motor neurons that make up the corticospinal tract, which transmits commands from the brain to the muscles, and the lower motor neurons that control the skeletal muscles by instructions from the brain. The signals from the brain are not transmitted to the muscles, leading to the gradual weakening of muscle strength and eventual muscle deterioration motor [1].

On the other hand, spasticity is seen to be a positive feature of upper motor neuron syndrome [1]. This is because it is due to a loss of inhibition of the lower motor neuron pathways, rather than a loss of connection to the lower motor neuron. This results from disordered sensorimotor control of movement due to a lesion of the upper motor neuron which regulates muscle control. Therefore, there is an imbalance of the signals between the Central Nervous System (CNS) and muscle, presenting as intermittent or sustained involuntary activation of muscles. Spasticity is common in ALS and can negatively affect coordinated movement, making activities of daily living difficult or unmanageable. Baclofen and tizanidine are commonly used to treat spasticity, although there is limited evidence supporting either in alleviating ALS-specific spasticity symptoms. In cases of refractory spasticity in which baclofen and tizanidine are ineffective, injections of botulinum toxin type A have a therapeutic effect. Botulinum toxin treatment can decrease spasticity and muscle tone for up to 90 days after injection with limited adverse effects [2-4]. Dosage and injection site is dependent on the location of spasticity, and this treatment is not commonly used in ALS because it may increase weakness.

ALS is a progressive illness with widespread systemic effects and persistent symptoms [1]. In the world, approximately 400,000 people have ALS. No effective treatment is currently available, and thus the development of new treatments is crucial.

In these circumstances Stem Cell (SC) therapy has been proposed as a promising treatment option for ALS based on the potential effects on various pathogenic mechanisms through trophic and/or immunomodulatory support and, perhaps, by providing host neural cell replacement. Indeed, in the past two decades, transplantation of various SC products has been evaluated in numerous Phase I and II clinical trials designed to assess feasibility and safety but also looking for indications of clinical benefit, reflected by changes in the progression rate of the ALS Functional Ratings Score (ALSFRS) and the respiratory function (forced or slow vital capacity) [5-7]. However, unfortunately, most clinical trials did not meet the main goal of showing the improvement of ALS symptoms.

The previous experimental studies showed that the transplantation of various types of stem cells and their derivatives, including human Bone Marrow Mesenchymal Stem Cells (BMMSC) adipogenic stem cells, placenta-derived stem cells, and neural precursor cells, promotes functional recovery in animal models of ALS [8-13]. However, the results of most studies indicate that the engrafted cells undergo minimal differentiation *in vivo* and survive for only short periods. In addition, recent studies showed that Mesenchymal Stem Cells (MSC) infused peripherally are prevented from infiltrating into the CNS and instead act peripherally to inhibit CNS inflammation. Several lines of evidence suggest that transplanted MSC can protect neural tissue through cell paracrine mechanisms [14,15].

Stem Cells from Human Exfoliated Deciduous Teeth (SHED) which is a family of MSC is derived from normal dental pulp. They are thought to originate from the cranial neural crest and express early mesenchymal and neuroectodermal stem cell markers [16]. In addition, they can differentiate into functional neurons and oligodendrocytes under the appropriate conditions SHED exhibit immunomodulatory and regenerative activities and have the potential to treat various diseases [11-16]. We showed previously that SHED transplantation into a completely transected rat spinal cord resulted in the functional recovery of hind limb locomotion [17]. Moreover, SHED engraftment promotes functional recovery from various acute and chronic CNS insults through paracrine mechanisms that activate endogenous tissue-repairing activity [18,19]. Furthermore, these studies showed that SHED-Conditioned Medium (SHED-CM) exerts similar therapeutic effects to those of SHED, including remyelination of the CNS.

Shimojima reported that SHED-CM immunomodulatory and regenerative activities have the potential to treat Experimental

Autoimmune Encephalomyelitis (EAE) [20]. In the case of EAE mice, it showed that microglia/macrophage activation induces inflammatory demyelination and subsequent neural damage in spinal code and results in a range of clinical features in sensory and motor paralysis, blindness, pain, incontinence, and dementia. Although the true cause of ALS is unknown, however, ALS has similar pathological and clinical features to EAE.

We made a general judgment that it was worth applying SHED-CM for treating ALS patients.

Here we report the efficacy of SHED-CM in treating a case with ALS. The patient treated with transnasal and intravenous administration of SHED-CM exhibited significantly improved ROM in limbs, reduced deterioration of pulmonary function. Our data suggest that SHED-CM may be a novel treatment for ALS.

Case Presentation

Patient history

A 68-years-old man was admitted to Yokohama city hospital in April 2019 due to progressively lower and upper limb weakness with muscle atrophy, which he suffered from for more than 10 months. He began to notice fasciculations in his left arm in June 2019 which then progressed to involve his whole right arm, chest, back, and eventually even the lower limbs. He also began to experience painful cramps in these locations in November 2019. After several examinations including EMG in head and neck muscle, MRI for the spinal cord, and Pulmonary Function Test (PFT) he was finally diagnosed with ALS in April 2020. With his diagnosis confirmed by a specialist, the patient could not accept that there was no treatment for ALS, so he ventured out on his own and began to research his condition and searched for any treatments that could return his life to normal; that is when he discovered SHED-CM Therapy.

Diagnosis with EMG, MRI, and PFT

He was referred and visited our clinic on January 2021, and we started to reevaluate many examination data performed in the other hospitals to diagnose his disease. First, we investigated EMG examinations performed in April 2019. Repetitive Nerve Stimulation (RNS) test at 3 Hz on trapezius muscles revealed neurogenic changes. Single-Fiber Electromyography (SF-EMG) examination in trapezius muscles also showed acute neurogenic change (Figure1). However, in the other muscle, Triceps, Delt, there were no abnormal findings. Furthermore, in the same muscles, they performed a Multi-Motor Unit Potential (MUP) analysis which showed normal values for amplitude, duration, and polyphasic, excluding neurogenic and myopathic changes. However, in June 2020 there was extensive denervation in the cranial nerves area (Table 1) and thoracic spine area. According to the records of Pulmonary Function Test (PFT), the patient's respiratory abilities was getting worse in the past 6 months, such as Vital Capacity (VC) 82.1% (April 2020, Figure 2) , 66.5% (June 2020), and 46% (August 2020) but the Forced Expiratory Volume in 1 second (FEV1) was kept in the normal range. Fortunately, SpO2 was kept within the normal limit in room air. Computerized Tomography (CT) and brain Magnetic Resonance Image (MRI) of the brain and spine were normal.

Preparation of SHED-CM and treatment protocols

From the various examination, we detected the diagnosis for the patient as ALS and decided to try the SHED-CM therapy for this patient. SHED-CM was prepared as described previously [20]. In brief, exfoliated deciduous teeth (from 6 years to 12-years-old children) were obtained from the ethics committee of Smart Clinic Tokyo, Japan. All participants provided written informed consent. After separating the crown and root, the dental pulp was isolated and digested in a solution of 3 mg/ml Collagenase type I and 4 mg/ml Dispase for 1 h at 37°C. After three to nine passages, SHEDs at 70%-80% confluency were washed with PBS twice, and the culture medium was replaced with serum-free DMEM. After a 48-h incubation, the medium was collected and centrifuged for 3 min at 440 × g (gravity). The supernatants were collected and centrifuged for 3 min at 4°C and 17,400 × g. The resulting supernatants were used as SHED-CM in various treatments (Figure 3).

Treatment and outcome

We treated the patient with trans nasal administration of 10% of SHEDCM from January 2021 for 12 months and intravenous (4 ml/kg/time) administration of SHEDCM from September 2021 a week for consecutive weeks, obtaining a clinical improvement in the strength of muscle and stable pulmonary function. In particular, after the first cycle of intravenous SHEDCM therapy, the patient showed the improvement of ROM in limbs and neck (Table 2). He showed clinical stability in ALS Functional Rating Scale-Revised Scale (ALSFRS-R) as 12/48 and an increase in range of motion (Figure 4). The SpO_2 was kept stable in the range of over 95% in room air. However, this improvement could not be evident in the legs and arms muscle groups, subsequently, he could not walk and move his arms and shoulder. Therefore, we repeated cyclically this treatment following 4 months obtaining the muscle strength. There were no adverse effects relating to SHED-CM therapy. As a special note, he suffered from mild aspiration pneumonia at the end of November 2021, then he received a tracheostomy just in case. However, he recovered quickly and continued treatment. In our treatment process, he received only one time of intravenous administration of SHED-CM exhibited significantly reduced disease severity in ROM and stability in pulmonary function and ALSFRS-R. These data suggested that the continuous injection of SHED-CM during the late stage of ALS can result in sustained improvements in its pathology.

Discussion

Currently, there is no known cure for ALS, but stem cell-based therapies may give patients, their doctors and scientists hope in dealing with this condition. ALS affects almost exclusively the motor nervous

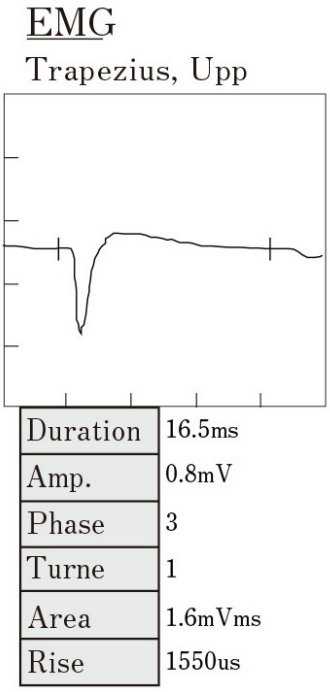

Figure 1. EMG in Trapezius.

Table 1. EMG summary in cranial nerve area.

EMG Summary Table	At Rest				Voluntary Contraction					Judge
Muscle	Fib	PS	MD	mld	Nm	LD	SD	Po	mV	-
R. Tongue	///	///	///	///	2-1	1	0	0	5	Neurogenic
R. Masseter	0	0	0	0	2	1	0	1	5	Neurogenic
R. T3 paraspinal	0	0	0	0	2-1	1	0	1	4	Neurogenic

FI:Fibrillation potentials, PS: Positive sharp wave, MD:Myotonic discharge, mid:Myotonic Like Discharge
Nm:Normal unit, LD:Long duration potential, SD:Short duration; Po:Polyphasic Potential,
mV:Amplitude of abnormal potential
3:many, 2:several, 1:few, 0:none, ///:Not at rest

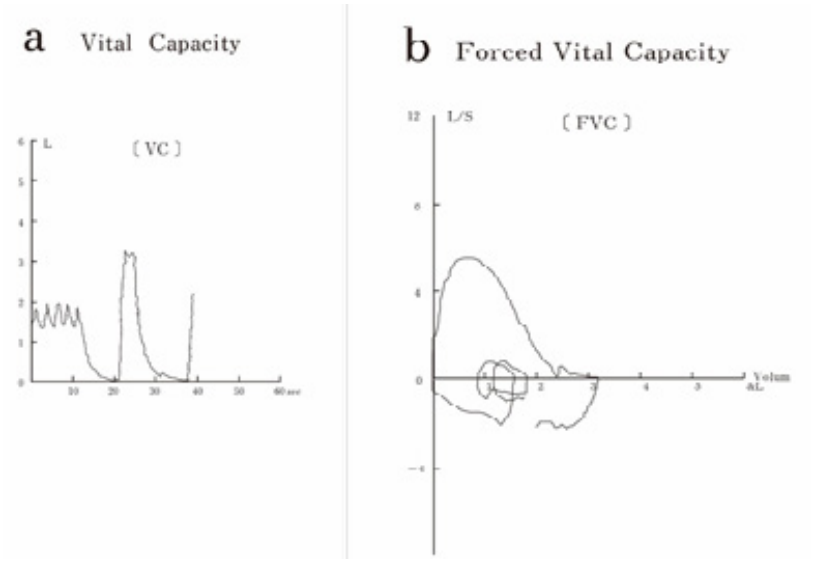

Figure 2. Lung Capacity (a. Vital capacity, b. Forced vital capacity).

Table 2. Passive & active range of motion before and after treatment.

Passive ROM			
Site		**Before**	**After**
Shoulder forward flexion	Right (R)	30	50
	Left (L)	40	60
Shoulder abduction	(R)	25	35
	(L)	25	30
Elbow flextion	(R)	50	90
	(L)	40	70
Forearm supination	(R)	-35	-5
	(L)	-45	-5
Wrist extension	(R)	0	10
	(L)	-45	10
Hip flextion	(R)	40	50
	(L)	35	60
Hip abduction	(R)	10	25
	(L)	15	25
Hip external rotation	(R)	10	20
	(L)	10	20
Knee flextion	(R)	70	80
	(L)	70	85
Active ROM			
Site		**Before**	**After**
Cervical rotation	(R)	0	30

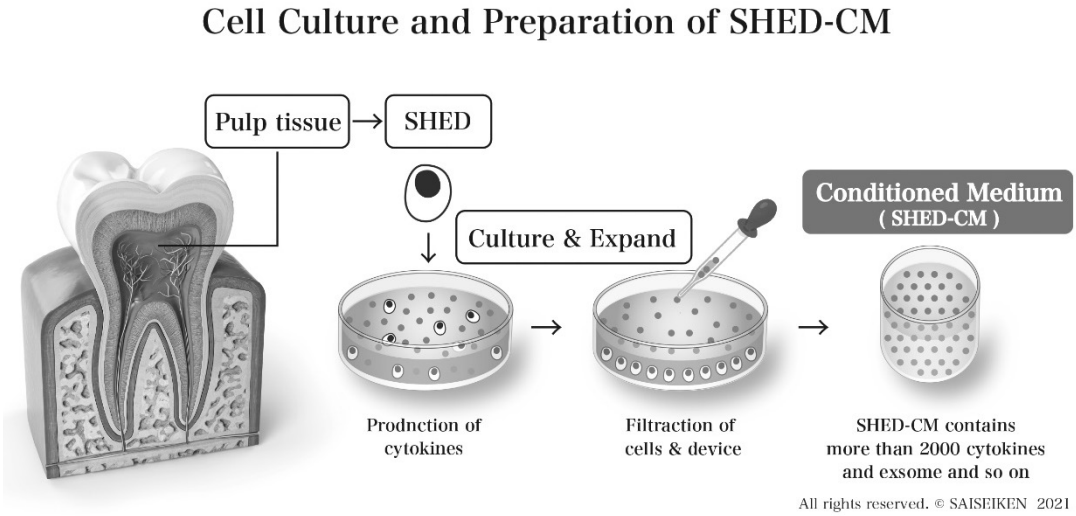

Figure 3. Preparation of SHED-CM.

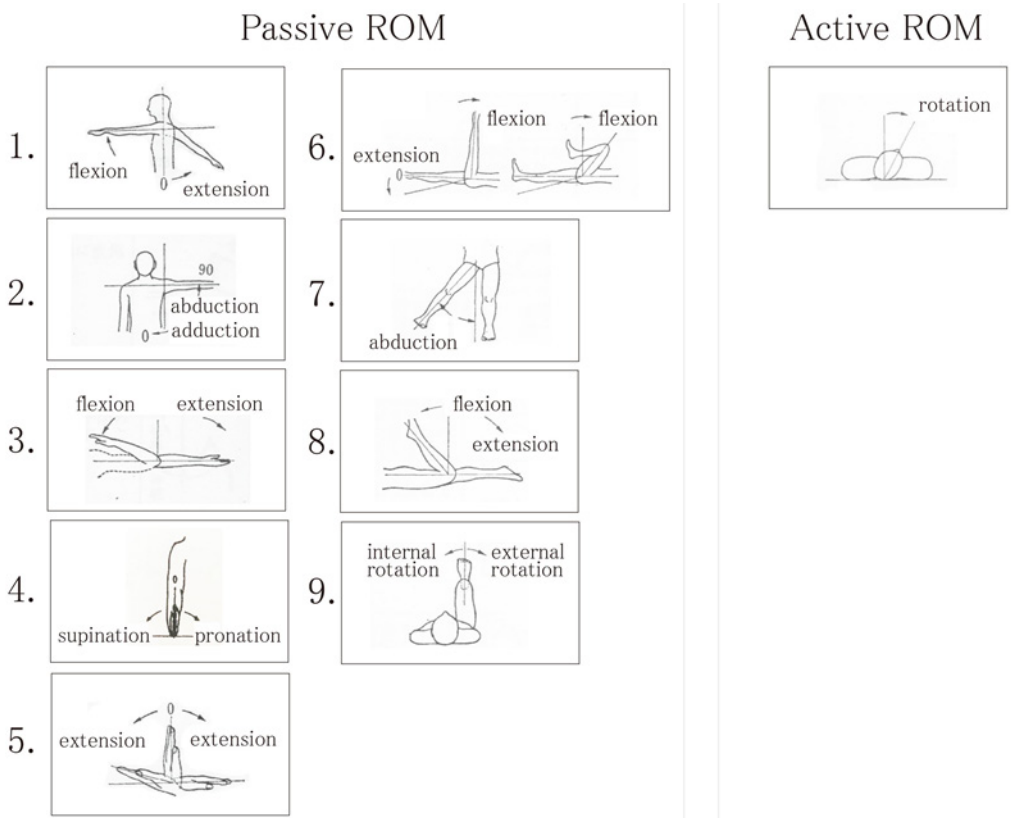

Figure 4. Diagrammatic representation of Active and Passive Range Of Motion (ROM).

system. This means that the system which controls the muscles and movements is impaired in patients with ALS. The sensation of touch, pain, and temperature seeing, hearing, smelling, and tasting, the functions of the bladder and intestine remain normal in most cases [1]. Once motor nerve cells in the spinal cord and their extensions to the muscles are affected, the disease leads to involuntary If the motor nerve cells in the brainstem are affected, muscles that control speech, chewing, and swallowing are weakened. This form of ALS is also called progressive bulbar paralysis. The disease of the motor nerve cells in the cortex and their connections to the spinal cord lead to both muscle paralysis and an increase in muscle tone (spastic paralysis) with an increase in reflexes. Unfortunately, there is no effective treatment and drug for such neurodegenerative symptoms.

Recent research has focused on the disease-modifying effects of Stem Cell (SC)-secretome. Although it is still unclear which ingredients of the SC-secretome are responsible for its effectiveness in ALS, several bioactive compounds have been identified, such as Brain Derived Neurotrophic Factor (BNDF), Glial Cell-Derived Neurotrophic Factor (GDNF), Nerve Growth Factor (NGF), Insulin-Like Growth Factor 1 (IGF-1), and Vascular Endothelial Growth Factor (VEGF), amongst others. These compounds have the potential to

- Exert a neuroprotective effect
- Reduce apoptosis (=programmed cell death)
- Increase the secretion of neurotrophic factors by glial cells (the cells in the brain which support neurons)
- Reduce oxidative stress
- Modulate the inflammatory process in the brain (stimulation of M2 cells that liberates anti-inflammatory bio compounds)

Moreover, due to our current research SHED-CM also contains a variety of growth factors, including neurotrophic factors, which promote such effects as neuroprotection, axonal elongation, neurotransmission, and immunosuppression [21-23]. Previous reports showed that several factors, including indoleamine 2,3-dioxygenase, IL-6, PGE2, LIF, and HGF, contribute to the immunosuppressive effects. HGF, in particular, is thought to be the critical factor in human SHED that promotes tissue regeneration and drives the antiproliferative effect on T cells [24]. HGF is also one of the most abundant components of SHED-CM, by cytokine Ab array analysis; however, our previous study identified ED-Siglec-9 as another major component of SHED-CM. Furthermore, we showed that ED-Siglec-9 and MCP-1 in SHED-CM could synergistically alter the M1/M2 polarity of macrophages/microglia *in vivo* [20]. Although the effect of SHED-CM on EAE depends on ED-Siglec-9, SHED-CM-treated mice showed somewhat better recovery than ED-Siglec-9-treated ones, suggesting that the other factors in SHED-CM, including HGF, might cooperatively improve EAE and ALS. There is also the possibility of spontaneous healing of motor neuron disease. There are a few reports that ALS may be reversible, even without therapy [25-27]. The temporal association of the improvements with the therapy in the case of our patient, however, argues against spontaneous healing. In addition, several other neurodegenerative diseases such as Alzheimer's disease and Stroke have improved considerably after therapy initiated by us based on a similar protocol. Therefore, we are confident that the patient had indeed suffered from motor neuron disease and that his healing was a consequence of the therapy. Our available scientific knowledge [19-23] with regards to the efficacy of SHED-CM-secretome in animal models that are suffering from a neurodegenerative disease like ALS encourages the development of cell-free products that can potentially eliminate the need for cell transplantation strategies in the future. Current research also indicates that SHED-CM-secretome is effective and safe in ALS patients, albeit still experimental treatment for ALS disease. Despite the lack of larger clinical trials, the available scientific data support the use of SHED-CM-secretome as a promising new treatment for ALS disease that improves the clinical status of patients, slows the disease progression, and improves the Quality Of Life (QOL). Spasticity can have an impact on an individual's function, affecting upper and lower limbs, as well as the trunk. If this is not managed effectively, it can lead to fixed deformity contractures and changes to soft tissue, which affect skincare, comfort, and hygiene, as well as complications for daily QOL [28].

We succeeded in the clinical improvement of ROM in limbs just 1 week after starting intravenous administration of SHEDCM and releasing the spasticity contracture. It was extremely helpful for the nursing care staff and family of the patient which can recover the patient's Activities of Daily Living (ADL).

Conclusion

In conclusion, we showed that the cell-free therapy using SHED-CM for ALS was possible which was the first human case treated by administration of SHED-CM. The therapy of SHED-CM significantly improved ALS patients QOL and ADL by releasing the spasticity contracture in limbs. The results of these and further studies may provide new cell-free therapies instead of conventional stem cell therapy for ALS.

References

1. Longinetti, E. & Fang, F., "Epidemiology of amyotrophic lateral sclerosis: An update of recent literature." *Curr Opin Neurol* 32.5 (2019): 771.
2. Marquardt, G. V., "Use of intrathecal baclofen treatment of spasticity in ALS." *J Neurol Neurosurg Pasychiatry* 72 (2002): 274-280.
3. Kamen, L., et al. "A practical overview of tizanidine use for spasticity secondary to multiple sclerosis, stroke, and spinal cord injury." *Curr Med Res Opin* 24.2 (2008): 425-439.
4. Marvulli, R., et al. "Botulinum toxin type A and physiotherapy in spasticity of the lower limbs due to amyotrophic lateral sclerosis." *Toxins* 11.7 (2019): 381.
5. Nabavi, S. M., et al. "Safety, feasibility of intravenous and intrathecal injection of autologous bone marrow derived mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: An open label phase I clinical trial." *Cell J* (Yakhteh) 20.4 (2019): 592.
6. Petrou, P., 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 Neurol* 73.3 (2016): 337-344.
7. Syková, E., et al. "Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: Results of phase I/IIa clinical trial." *Cell Transplant* 26.4 (2017): 647-658.
8. Mazzini, L., et al. "Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial." *Exp Neurol* 223.1 (2010): 229-237.
9. Uccelli, L. M. & Vito, P., "Mesenchymal stem cells in health and disease." *Exp Neurol* 8.9 (2008): 726-736.
10. Uccelli, A. & Darwin, J. P., "Why should Mesenchymal Stem Cells (MSCs) cure autoimmune diseases?" *Curr Opin Immunol* 22.6 (2010): 768-774.
11. Yousefi, F., et al. "Comparison of in vivo immunomodulatory effects of intravenous and intraperitoneal administration of adipose-tissue mesenchymal stem cells in Experimental Autoimmune Encephalomyelitis (EAE)." *Int Immunopharmacol* 17.3 (2013): 608-616.
12. Fisher-Shoval, Y., et al. "Transplantation of placenta-derived mesenchymal stem cells in the EAE mouse model of MS." *J Mol Neurosci* 48.1 (2012): 176-184.
13. Rooney, J., et al. "What does the ALSFRS-R really measure? A longitudinal and survival analysis of functional dimension subscores in amyotrophic lateral sclerosis." *J Neurol Neurosurg Psychiatry* 88.5 (2017): 381-385.
14. Berry, J. D., et al. "NurOwn, phase 2, randomized, clinical trial in patients with ALS: Safety, clinical, and biomarker results." *Neurology* 93.24 (2019): 2294-2305.
15. Gordon, David, et al. "Human mesenchymal stem cells infiltrate the spinal cord, reduce demyelination, and localize to white matter lesions in experimental autoimmune encephalomyelitis." *J Neuropatho Exp Neurol* 69.11 (2010): 1087-1095.
16. Kiraly, M., et al. "Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons." *Neurochem Int* 55.5 (2009): 323-332.
17. Sakai, Kiyoshi, et al. "Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms." *J Clin Investig* 122.1 (2012): 80-90.
18. Drago, D., et al. "The stem cell secretome and its role in brain repair." *Biochimie* 95.12 (2013): 2271-2285.
19. Matsubara, K. et al. "Secreted ectodomain of sialic acid-binding Ig-like lectin-9 and monocyte chemoattractant protein-1 promote recovery after rat spinal cord injury by altering macrophage polarity." *J Neouroscience* 35 (2015): 2452-2464.
20. Shimojima, C., et al. "Conditioned medium from the stem cells of human exfoliated deciduous teeth ameliorates experimental autoimmune encephalomyelitis." *J Immunol* 196 (2016): 1-8.
21. Mita, T., et al. "Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer's disease." *Behav Brain Res* 293 (2015): 189-197.
22. Inoue, T., et al. "Stem cells from human exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats." *Tissue Eng* 19.1-2 (2013): 24-29.
23. Yamagata, M., et al. "Human dental pulp-derived stem cells protect against hypoxic-ischemic brain injury in neonatal mice." *Stroke* 44.2 (2013): 551-554.
24. Bai, L., et al. "Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models." *Nat Neurosci* 15.6 (2012): 862-870.
25. Tucker, T., et al. "Subacute, reversible motor neuron disease." *Neurology* 41.10 (1991): 1541-1541.
26. Tsai, Ching-Piao., et al. "Reversible motor neuron disease." *Eur Neurol* 33.5 (1993): 387-389.
27. Vale, T. C., et al. "Reversible lower motor neuron disease: A new case of a forgotten disease." *J Neurol Re.* 3.1 (2013): 40-41.
28. Simmons, Z., "Patient-perceived outcomes and quality of life in ALS." *Neurotherapeutics* 12.2 (2015): 394-402.

Cite this article: Ueda M, Seta Y. The First in Human Case of Amyotrophic Lateral Sclerosis Treated with Stem Cell-Derived Conditioned Medium: A 1-Year- Follow Up. Neurol Neurorehabilit. 2022, 4 (2), 011-0015.