Multiple doses of cell therapy and neurorehabilitation in amyotrophic lateral sclerosis: A case report

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Abstract
Cell therapy, along with intensive rehabilitation has been shown to significantly improve outcomes in amyotrophic lateral sclerosis (ALS), in addition to standard therapy. We present a 40-years-old male ALS patient, suffering for the past four years, who underwent multiple doses of cell therapy at our institution. Along with riluzole treatment and lithium co-administration, his treatment involved multiple intrathecal transplants of autologous bone marrow-derived mononuclear cells, followed by multidisciplinary neurorehabilitation. The outcome measures of ALS-Functional Rating Scale Revised score remained stable, and importantly, Six Minute Walk Test distance improved from 475.2 m to 580.8 m, over a span of 16 months. Improved outcomes are indicative of slowing down of disease progression. Multiple doses of intrathecal autologous cell therapy along with rehabilitation and lithium, in addition to standard riluzole treatment is a novel approach for decelerating disease progression and qualitatively improving living conditions for ALS patients and their caregivers.

Introduction
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that selectively targets upper and lower motor neurons, and is clinically characterized by wasting, fasciculating musculature that is eventually fatal. Prognosis depends upon numerous factors, such as type of ALS, gender, and age of symptom onset.1 Thorough neurological and electrophysiological investigations are required to confirm the diagnosis of ALS on the basis of the revised EL Escorial Criteria for ALS diagnosis.2 Worldwide, the annual incidence of ALS is approximately 1-2.6 cases per 100,000.3 ALS treatment demands a comprehensive approach for slowing down disease progression and improving quality of life due to the multifarious pathophysiology of ALS. Studies implicate a variety of genetic mutations, misfolded protein aggregates, excessive free radicals, and excitotoxic agents in the pathology of ALS. Current pharmacological treatment strategies only provide symptomatic relief: riluzole curbs glutamate excitotoxicity; dextromethorphan/quinidine treats pseudobulbar affect; and edaravone scavenges free radicals, but only in the early disease stages.4 Multidisciplinary rehabilitation, respiratory management, assistive devices, adaptive equipment and an experienced rehabilitation team at each disease stage are currently the standard of ALS care.5 There is an urgent need worldwide to formulate safe, effective, and holistic treatment options for patients living with ALS that can significantly slow down or reverse the disease pathology.

Autologous bone marrow-derived mononuclear cells (BMMNCs) have proven to be a safe and effective therapeutic intervention for treating neurological disorders including ALS.6,7 Transplanted cells tend to integrate better into target tissue when a rehabilitative regimen follows the transplant.8-10 We present a case of 40-year-old male with Definite ALS, who underwent multiple intrathecal autologous BMMNC transplants with lithium and standard treatment at our institution. In this unique case, we are evaluating 6 Minute Walk Test (6MWT) scores along with ALS-Functional Rating Scale Revised (ALSFRSr). To our knowledge, there are no previous studies that show therapeutic effects of this therapy on the 6MWT in ALS.

Case Report
A 40-year-old male, with no family history of ALS or psychological disorders, noticed weakness in the right wrist in April 2014. Fasciculations started by June 2014, bilaterally in upper and lower limbs. Muscle cramps were noted in neck and legs. By September 2014, forearm muscle weakness ensued, along with left wrist weakness. At this point in time, he underwent an Electromyography (EMG) study, which showed diffuse denervation and reinnervation of multiple upper limb muscles with no conduction block.

A repeat EMG and Nerve Conduction Study (NCS) in December 2014 detected lower limb fasciculations. By January 2015, he started showing proximal muscle weakness in bilateral upper limbs. There were no facial or tongue fasciculations, dysphagia, dysarthria, sialorrhea, or dyspnea. Lower limb muscle strength was intact. Despite being treated with riluzole, supplements, rehabilitation, and undergoing hyperbaric oxygen therapy, his condition deteriorated.

Magnetic resonance imaging (MRI) using diffusion tensor imaging of the brain was normal. MRI cervical spine revealed mild/moderate spinal canal stenosis at C5-6. EMG and NCS showed evidence of a diffuse progressive disorder of motor neurons or their axons, and this was most severely affecting the cervical segments.

On assessment in November 2016, ALSFRSr score was 40/48; Functional Independence Measure (FIM) score was 109/126; and, 6MWT distance was 475.2 m. Upper motor neuron involvement manifested as hyperreflexia in knee jerk reflexes bilaterally in lower limbs; lower motor neuron signs were evident with hypotonia of
the upper limbs, subluxation of shoulders, muscle atrophy and depressed deep tendon (biceps, triceps, and supinator jerk) reflexes; and bilateral wasting of pectoral, scapular, forearm, thenar and hypothenar muscles. Muscle weakness was also observed in the neck. Additionally, he noticed cramping in both upper and lower limbs. Bilateral gastrocnemius-soleus and hamstring tightness, and weakness in upper extremities was noted. He also underwent psychological evaluation and had normal cognitive function. There was no family history and no genetic tests were done.

Another motor neuron disease, progressive muscular atrophy (PMA), was considered in the differential diagnosis; but he was eventually diagnosed as Definite ALS due to upper (hyperreflexia) and lower (hypotonia) motor neuron signs in multiple segments of the body according to the revised El Escorial criteria.2 His chief complaints at assessment included difficulty in performing instrumental activities of daily living (ADLs), like writing, typing, driving, cutting food, gripping and lifting objects; fine motor ADL movements like buttoning shirts, tying shoelaces; overhead movements such as those involved in bathing, transition activities such as getting up from the bed, and fatigue.

The patient reported minimal neck pain, but no pain radiating from the neck into the upper limbs. He also observed transient muscular chest pain.

Neuroregenerative therapy

Pre-intervention

The Institutional Ethics Committee provided the ethical approval for this treatment; it was in concordance with the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects.11 A written informed consent was obtained from the patient after explaining the procedure in detail. An experienced team of doctors and therapists thoroughly examined the patient. Anesthetic and surgical fitness was confirmed via pre-surgical routine blood tests, urinalysis, and chest X-ray.

Bone marrow aspiration

Forty-eight hours and 24 hours prior to autologous BMMNCs transplant, 300 μg of Granulocyte-Colony Stimulating Factor (G-CSF) injections were administered subcutaneously, as G-CSF mobilizes BMMNCs from their niche, stimulates CD34+ cells and increases their survival and multiplication rate.12 Local anesthesia was administered in the region of anterior superior iliac spine; 110 mL of bone marrow was aseptically aspirated, collected in heparinized tubes, and transferred to regenerative medicine laboratory.

Separation of autologous bone marrow-derived mononuclear cells

BMMNCs were separated from the aspirated bone marrow by aseptic differential centrifugation. The BMMNCs, obtained as a separate layer, were washed, resuspended in normal saline, and counted. Viability estimate of the isolated BMMNCs was obtained using Trypan blue vital dye on a hemocytometer for total and viable count. 1.2×10^6 BMMNCs were obtained at a viability rate of 98%. This was further confirmed by TALI cell counter. CD34+ analysis was done using Fluorescence Activated Cell Sorting using CD34 PE antibody, and the CD34+ fraction was found to be 5.38%.

Administration of autologous bone marrow-derived mononuclear cells

Immediately post separation, isolated BMMNCs were administered intrathecal by L4-L5 level; simultaneously, 1 gm methylprednisolone in 500 mL Ringer’s Lactate solution was injected intravenously to reduce local inflammation. The patient was closely monitored for any immediate adverse events during his stay at the hospital.

Neurorehabilitation therapy

In physiotherapy, the patient was administered strength training exercises for upper limbs, lower limbs and trunk; sitting and standing balance training exercises, gait training, and respiratory exercises to improve lung capacity and stamina; bed mobility exercises; and energy conservation techniques. Exercises to improve fine and gross motor skills of upper limbs, and ADL retraining were done as part of occupational therapy. Exercises to improve fine and gross motor skills of upper limbs, and ADL retraining were done as part of occupational therapy. Additionally, psychological and family counseling was performed to help the patient and his family to cope with the difficult diagnosis of ALS. Post discharge, he was put on a home exercise program.

Table 1. Symptomatic analysis of disease progression depicts a stable condition.

<table>
<thead>
<tr>
<th>Measurements or symptoms</th>
<th>At assessment</th>
<th>At 4 months after 1st transplant (just before 2nd transplant)</th>
<th>At 10 months after 1st transplant (just before 3rd transplant)</th>
<th>At 16 months after 1st transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulation</td>
<td>Ambulatory with some difficulties</td>
<td>Maintained</td>
<td>Maintained</td>
<td>Maintained. Stamina has improved. He can perform 25 mins of brisk walking on the treadmill at inclination level 3, at 5.5 km/h</td>
</tr>
<tr>
<td>Leg movements</td>
<td>Difficulty during ambulation</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
</tr>
<tr>
<td>Maximum inspiratory volume (mL)</td>
<td>540</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Peak expiratory flow rate (L/min)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Chest expansion (in cm)</td>
<td>Upper 5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Middle 6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lower 6.5</td>
<td>6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Stamina/Level of fatigue</td>
<td>Fatiguability present</td>
<td>Same as before</td>
<td>Same as before</td>
<td>Improved walking stamina and exercising ability</td>
</tr>
<tr>
<td></td>
<td>Reach test (in inches)</td>
<td>Forward 6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Backward 6.5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left 7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right 7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
Medical management

He was prescribed to continue 50 mg riluzole twice a day. In addition, 300 mg lithium was prescribed once a day, and serum lithium levels were maintained at 0.5 to 0.8 mmol/L for 6 weeks.

Based on improvements seen after the first transplant (Tables 1 and 2; Figures 1 and 2), the patient underwent a second transplant four months later (1.6×10⁸ total autologous intrathecal BMMNCs; cell viability-98%; CD34⁺ fraction-2.06%), and a third transplant 10 months after the first transplant (3.5×10⁸ total autologous intrathecal BMMNCs; cell viability-98%; CD34⁺ fraction-3.02%). Each transplant was followed by a comprehensive neurorehabilitative routine as described above. The serial scores on different outcome measures were evaluated and compared to natural disease progression to understand the effect of the treatment.

Post-transplant, his gross motor skills improved. At four months (second transplant) and 10 months (third transplant) since the first transplant, his ambulation, stamina and pulmonary function remained stable. At 16 months since the first transplant, his ambulation improved, owing to increased lower limb strength. His pulmonary function improved, as did his stamina and reach. His condition was otherwise maintained. Functionally, he still requires some assistance in his activities of daily living. However, his fine motor skills and overhead activity showed some deterioration.

Symptomatic analysis of the patient over the course of 16 months was performed (Table 1). Improvements in the patient’s outcome measures were charted, depicting his stability over 16 months (Table 2; Figures 1 and 2).

The FIM scores of the patient dropped from 109 to 99 over 16 months. This change was observed primarily in the motor section of the FIM scale. This patient reports to have some deterioration only in his distal upper limbs, possibly due to lack of upper limb exercises, and his disease had not progressed significantly. FIM is limited only to functional independence and is not a reliable measure of disease progression in this case.

Discussion and Conclusions

Numerous clinical publications advocate the safety and efficacy of adult autologous BMMNCs as a therapy for neurological disorders, including ALS.⁶¹³ No irreversible adverse events have been reported post transplant. These cells are a safe treatment modality because they are autologous adult cells, do not elicit an immune response,¹⁴ and have no tumorigenic properties or ethical issues. The intrathecal delivery of cells diluted in autologous cerebrospinal fluid is particularly attractive, because it is a minimally invasive procedure that facilitates efficient homing of BMMNCs across the blood-brain barrier in a relatively immune-privileged environment.¹⁵

Lithium improves the survival and potency of BMMNCs, as well as their integration into target tissue,¹⁶ while being well tolerated by ALS patients.¹⁷ We have also published a study that showed that as compared to controls there was higher survival (by 30.38 months) of ALS patients who underwent intrathecal autologous BMMNC transplant followed by six weeks of

Table 2. Changes in outcome measures over 16 months.

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>At assessment before 1st transplant</th>
<th>At 4 months after 1st transplant (just before 2nd transplant)</th>
<th>At 16 months after 1st transplant (6 months after 3rd transplant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT (in m)</td>
<td>475.2</td>
<td>514.8</td>
<td>580.8</td>
</tr>
<tr>
<td>FIM (out of 126)</td>
<td>109</td>
<td>104</td>
<td>99</td>
</tr>
<tr>
<td>ALSFRSr (out of 48)</td>
<td>40</td>
<td>40</td>
<td>38</td>
</tr>
</tbody>
</table>

Figure 1. The patient’s 6 Minute Walk Test (6MWT) distance improved as time progressed post intervention. 6MWT distance in the asymptomatic state is the median distance obtained from values measured by Sanjak et al.²³ in healthy subjects aged 50 to 85 years, measured in meters.

Figure 2. Data points show score values measured at different intervals. Initial points indicate asymptomatic scores for each outcome, i.e. complete functionality. ALS-Functional Rating Scale Revised is scored out of a total of 48 points, and FIM is scored out of 126 points.
lithium. Subgroup analysis showed that the patients who received lithium showed a clinically significant higher mean survival of 106.73 (±15.69) months as compared to the ones that did not receive lithium, 66.83 (±7.52) months (P=0.121). However, 6MWT was not used in this study.

BM-MNCs reactivate the degenerating nervous system by migrating and differentiating into different cell types in vivo. These cells also regulate cellular microenvironments by the modulation of cytokines like vascular endothelial growth factor (VEGF), ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF), platelet-derived growth factor-BB (PDGF-BB), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α and interleukins (IL-1α, IL-β, IL-6, IL-10). Rehabilitation and exercise post transplantation have several neurological merits. Exercise at moderate intensity increases synaptogenesis and dendritic branching in multiple brain regions, in addition to BDNF, IGF-I, and NGF secretion. Conversely, lack of exercise impedes endogenous neurogenesis and downregulates neurotrophic factor production. Further, several studies attest to the beneficial effects of moderate exercise in ALS patients.

Prabhakar et al. monitored the disease progression for a period of 12 months using ALSFRSr of 10 ALS patients treated with autologous BM-MNCs intrathecally. They show that a four-point deterioration on the ALSFRSr scale took a median of 16.7 months. Here, the ALSFRSr score decreased by a mere two points in a span of 16 months post intervention. 6MWT is a pertinent monitor of ALS-related decline. Sanjak et al. demonstrate that the mean walking distance ranges from 576 m to 631 m during 6MWT (median distance=603.5 m; see Asymptomatic 6MWT data point in Figure 1), in healthy subjects aged 50 to 85 years. They also report that as the disease progressed, the distance covered by ALS patients reduced significantly. Here, the patient’s 6MWT distance increased from 475.2 m at pre-assessment to 580.8 m at 16 months. The 6MWT provides an additional, robust measure of cardiopulmonary function, and conurs with the inference derived from the ALSFRSr scores.

This case report demonstrates the stabilization of a male Definite ALS patient via multiple doses of cell therapy and lithium co-administration, along with standard riluzole treatment and neurorehabilitation. Autologous BM-MNCs along with lithium and standard treatment may have mitigated disease progression, and improved the condition of this patient. Randomized controlled clinical trials with scientific rigor and a large sample size are required for deriving conclusive evidence and standardizing this therapeutic regimen as treatment for this devastating disorder.

References