Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up

H. Deda a, Mc Inci a, Ae Kürekçi b, A. Sav c, K. Kayhan c, E. Öğün c, Ge Üstünsoy c, S. Kocabay e

a Department of Neurosurgery and Neurology, Akay Hospital, Ankara, Turkey
b Department of Pediatric Hematology, GATA, Ankara, Turkey
c Department of Pathology, University of Marmara, Ankara, Turkey
d WSC Inc., Ankara, Turkey
e Sla Neurorehabilitation Center, Ankara, Turkey

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Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up

H Deda, MC Inci, AE Kürekçi, A Sav, K Kayhan, E Özgün, GE Üstünsoy and S Kocabay

1Department of Neurosurgery and Neurology, Akay Hospital, 2Department of Pediatric Hematology, GATA, 3Department of Pathology, University of Marmara, 4WSC Inc., and 5Sıla Neurorehabilitation Center, Ankara, Turkey

Background
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive loss of spinal cord and cortical motoneurons. Despite improved understanding of the mechanisms underlying ALS, in clinical practice the management of ALS remains essentially supportive and focused on symptom relief. However, over the past few years stem cell research has expanded greatly as a tool for developing potential new therapies for treating incurable neurodegenerative diseases.

Methods
Thirteen patients with sporadic amyotrophic lateral sclerosis (SALS) were included in this study, and bone marrow (BM)-derived hematopoietic progenitor stem cells were used. We selected patients with bulbar involvement and severe loss of movement. Our aim was to put the stem cells into the end of the brain stem and at the beginning of the spinal cord because the blood–brain barrier is intact in ALS and this region was the most affected part in our patients. Under general anesthesia, a total laminectomy was performed at the C1–C2 level. Stem cells were injected to the anterior part of the spinal cord.

Results
During the follow-up of 1 year after stem cell implantation, nine patients became much better compared with their pre-operative status, confirmed by electroneuromyography (ENMG). One patient was stable without any decline or improvement in his status. Three patients died 1.5, 2 and 9 months, respectively, after stem cell therapy as a result of lung infection and myocardial infarction (MI).

Discussion
These results show that stem cell therapy is a safe, effective and promising treatment for ALS patients.

Keywords
amyotrophic lateral sclerosis, bone marrow, repair, stem cell.

Introduction
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive loss of spinal cord and cortical motoneurons. Despite improved understanding of the mechanisms underlying ALS, in clinical practice the management of ALS remains essentially supportive and focused on symptom relief. Riluzole, an anti-glutamate agent, remains the only available treatment for ALS but has limited therapeutic benefits, with minimal effects on patient survival and no effect on muscle strength, quality of life or functional capacity [1,2].

Over the past few years, stem cell research has expanded greatly as a tool for developing potential new therapies to treat incurable neurodegenerative diseases. Stem cell transplantation has been effective in several animal models, but the underlying restorative mechanisms are still unknown [3–5]. Several mechanisms, such as cell fusion, neurotrophic factor release, endogenous stem cell proliferation and
transdifferentiation, may explain the positive therapeutic results, in addition to replacement of lost cells. The absence of any effective pharmacologic treatment and the preliminary data in both experimental and clinical settings has recently identified ALS as an ideal candidate disease for the development of stem cell therapy in humans [6–9].

**Methods**

**Patient selection and consent procedure**

Thirteen patients with sporadic amyotrophic lateral sclerosis (SALS) were included in this phase II study. The patients were operated on between January and March of 2006. The transplantation protocol was carried out with the support and approval of the regional ethics board (Akay Hospital Ethics Board, Ankara, Turkey). All documentation was sent to the Ministry of Health, and the stem cell committee of the Ministry of Health approved the results. Hence the results have been approved by an institutional review board.

Written informed consent was taken from the patients following appropriate procedures. The patients, who were tetraplegic and on ventilators, had the cognitive abilities to give such informed consent. In addition, all the informed consent procedures were recorded with video cameras and patients’ families and hospital staff signed the informed consents as witnesses.

Inclusion criteria for the selected patients were: (1) disease duration had been longer than 6 months; (2) ALS had been confirmed by ENMG on two separate occasions; (3) the patient had been receiving maximal medical therapy as directed by a qualified hospital neurology department; and (4) the patient’s neurologic condition was in rapid decline and vital capacities were in a terminal period, as suggested by mechanical ventilator dependence or the inability to swallow or speak. Exclusion criteria were: (1) the presence of a neurologic deficit not related to ALS (e.g. cervical spinal cord injury); (2) an anatomical myelomalasia of the cervical cord, as visualized by magnetic resonance imaging (MRI); and (3) any serious pre-existing medical conditions that would make treatment of the patient inappropriate.

All the patients were examined by the same medical doctor before and after the stem cell operation. In addition, the progress of all patients was evidenced and documented with video cameras.

**Separation of human bone marrow cells**

Bone marrow (BM; 100–150 mL) was aspirated from the iliac crest using a standard procedure and processed to obtain mononuclear cells (MNC). BM aspirates were drawn in medical facilities in Ankara (Turkey), packed in an insulated shipping container with a cold pack, and delivered to an international express courier for delivery to the laboratories of Aastrom Biosciences Inc. (Ann Arbor, MI, USA). All cell processing was performed under controlled conditions in Aastrom’s laboratories. Prior to processing, the BM aspirate was tested for sterility and cell numbers. The MNC were then separated for other components of BM using a Ficoll density gradient separation procedure. The resulting purified MNC were tested for cell viability, total cell number and CD34+ cell content. Following product testing the MNC were concentrated via centrifugation and resuspended in 6 mL of a 10% dimethylsulfoxide (DMSO) solution and frozen in a total of four cryovials in a controlled rate freezer. Frozen MNC were then shipped at −80°C via express courier to the hospital for administration to the patient.

**Operation**

Under general anesthesia, the patient was placed in a prone position and a total laminectomy was performed at the C1–C2 level to provide sufficient access to the site of the spinal cord. The dura was then incised and opened. The arachnoid was observed and opened with microscissors. If arachnoiditis was detected, a small part of the arachnoid was taken for biopsy. The dorsal surface of the spinal cord was cleared under high-power microscopic magnification. Bilateral dentate ligaments related to the laminectomy sites were cut with microscissors under a highly magnifying operative microscope. After exposure of sufficient surface on the spinal cord, avascular and safe areas were identified to inject the stem cells. Because stem cells were injected into the anterior part of the spinal cord, the depth of the injection site was measured. To avoid direct injury, 0.1 mL stem cells were injected using a 21-gauge needle attached to a 1-mL syringe to multiple different areas in the spinal cord. After this procedure, the spinal cord was covered with Gel Foam stem cell storage material and a piece of this material was settled on the lower cranial nerve and surface of the brain stem and a further 3 mL stem cells (at least 10 million cells) injected into it. The dura was closed and 1.5 mL (approximately 5 million cells) injected into the subarachnoid space at the
operation site, and at the same time 1.5 mL (approximately 5 million cells) stem cells were given intravenously by the anesthesiologist. The muscle and skin were closed layer by layer.

**Results**

The results of the patients are summarized in Tables 1 and 2. Post-operative bulbar scores and Norris scales were evaluated at 3 months. According to stem cell therapy, there were no complications; this was confirmed by MRI at 3 months (Figure 1). Pre- and post-operative ENMG were carried out for all patients to evaluate re-innervation; re-innervation was confirmed in seven patients. Each case is summarized below.

**Case 1**

A 54-year-old male was diagnosed with ALS in 2003. He was wheelchair-bound in 2005 and had been ventilating mechanically for 2 months prior to the study. Before stem cell therapy his neurologic findings were: muscle strength of upper and lower extremities, 1/5; head and neck control totally lost (bulbar score 6, Norris scale 21). Three weeks after the stem cell operation his neurologic findings were: muscle strength of upper and lower extremities, 2/5; body and neck muscle strength, 2/5. While sitting he could control his head and neck. During the day he could breathe by himself without any support; during sleep, because of panic attacks, he wanted to be on a mechanical ventilator. Three months later he could breathe by himself safely without any support, and his ENMG showed regeneration (bulbar score 9, Norris scale 39). His neurologic findings were stable at 1-year follow-up.

**Case 2**

A 60-year-old male was diagnosed with ALS in 2004. Six months after diagnosis he was wheelchair-bound and after 1 year he could not swallow or breathe by himself and needed mechanical ventilation. Before stem cell therapy his neurologic findings were: tetraplegic; no reaction in mimic muscles; total external ophtalmoplegia; bound to mechanical ventilation (bulbar score 0, Norris scale 3). Three weeks after the stem cell operation his neurologic findings were: although bound to a mechanical ventilator, some spontaneous breathing that was ineffective; eye movement intact; muscle strength at left and right m. frontalis, 2/5 and 1/5, respectively; strength of m. zygomaticus, 2/5. With these findings he could smile and...
express himself with movements. Three months later some of his neurologic findings had regressed slightly (bulbar score 2, Norris scale 7). One year later his neurologic findings were stable.

Case 3
A 54-year-old male was diagnosed with ALS in 2003. Before stem cell therapy his neurologic findings were: muscle strength of the left upper extremity 0/5, right upper extremity 2/5, lower extremities 3/5; he could walk with limited support. His talking and swallowing had been getting worse, especially in the 2 months prior to the study (bulbar score 7, Norris scale 54). Three weeks after the stem cell operation his neurologic findings were: muscle strength of the left upper extremity 1/5, right upper extremity 3/5, left lower extremity 3/5, right lower extremity 4/5; he could walk by himself easily. His talking and swallowing were better compared with the pre-operative period and his breathing was very good (bulbar score 11, Norris scale 68). His ENMG showed re-innervation potentials in his upper and lower extremities. One year later some of his findings had regressed a little but his neurologic findings were much better compared with the pre-operative period and he was stable.

Case 4
A 34-year-old female was diagnosed with ALS in 2000. Before stem cell therapy her neurologic findings were: muscle strength of the upper extremities 1/5, lower extremities 2/5; she could only walk with support. Her talking and swallowing were getting worse, especially in the 3 months prior to the study (bulbar score 6, Norris scale 48). Three weeks after the stem cell operation her neurologic findings were: muscle strength of the upper extremities 2/5, lower extremities 3/5; she could walk by herself easily. Her talking and swallowing were getting better compared with the pre-operative period and her breathing was very good (bulbar score 10, Norris scale 64). One year later her neurologic findings had regressed slightly but she was stable.

Case 5
A 50-year-old male was diagnosed with ALS in 2001. Before stem cell therapy his neurologic findings were: tetraplegic; mimic muscles intact; he could not breathe by himself nor swallow and was bound to mechanical ventilation (bulbar score 4, Norris scale 8). Three weeks after the stem cell operation his neurologic findings were: muscle strength at right m. biceps and right m. quadriceps 1/5 and

Table 2. Pre- and post-operative neurologic and laboratory findings of ALS patients

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</table>

*R, re-innervation; S, same; D, died.

Figure 1. Post-operative MRI showing that there was no complication as a result of stem cell implantation.
left hip muscle 1/5; although bound to a mechanical ventilator some spontaneous breathing that was ineffective and he could swallow (bulbar score 7, Norris scale 14). One year later his neurologic findings had slightly regressed but he was stable.

**Case 6**

A 34-year-old male was diagnosed with ALS in 2001. He could breathe by himself but it was not effective and he was borderline regarding requiring mechanical ventilation; he could not swallow. Before stem cell therapy his neurologic findings were: tetraplegic; mimic muscles intact; he could not talk (bulbar score 5, Norris scale 15). Three days after the operation, because of a lung infection, he was put on mechanical ventilation for 3 weeks but later he could breathe by himself without any mechanical help. Three weeks after the stem cell operation his neurologic findings were: muscle strength at lower extremities 1/5, bilateral hip muscle strength 2/5; swallowing was getting better (bulbar score 10, Norris scale 34). One year later his neurologic findings were stable and he could swallow easily.

**Case 7**

A 61-year-old female was diagnosed with ALS in 2003. Before stem cell therapy her neurologic findings were: muscle strength of the upper extremities 2/5, lower extremities 1/5. She could not walk and her swallowing had been getting worse, especially in the 3 months prior to the study (bulbar score 8, Norris scale 52). Three weeks after the stem cell operation her neurologic findings were almost the same. Six months later an electroneuromyography (EMG) showed re-innervation in her muscles but after 8 months, because of a lung infection, she needed mechanical ventilation; 1 month later she died as a result of severe lung infection.

**Case 8**

A 55-year-old male was diagnosed with ALS in 2003. Before stem cell therapy his neurologic findings were: head and neck control totally lost; muscle strength of the upper extremities 1/5, lower extremities 1/5. He could not walk, and his talking and swallowing had been getting worse, especially in the month prior to the study (bulbar score 2, Norris scale 34). Three weeks after the stem cell operation his neurologic findings were: muscle strength of the upper extremities 3/5, lower extremities 4/5. He could walk by himself easily; his talking and swallowing were getting better compared with the pre-operative period and his breathing was very good (bulbar score 6, Norris scale 62). Figures 2 and 3 show his pre- and post-operative EMG. Eight months later his neurologic findings had regressed slightly; 1 year later his neurologic findings were slightly worse but still better compared with the pre-operative period.

**Case 9**

A 41-year-old male was diagnosed with ALS in 2000. Before stem cell therapy his neurologic findings were: muscle strength of the upper extremities 1/5, lower extremities 2/5. He could only walk with support, and his talking and swallowing had been getting worse, especially in the 10 months prior to the study (bulbar score 6, Norris scale 49). Three weeks after the stem cell operation his neurologic findings were: muscle strength of the upper extremities 2/5, lower extremities 3/5. He could walk by himself easily; his talking and swallowing were getting better compared with the pre-operative period and his breathing was very good (bulbar score 9, Norris scale 50).

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**Needle EMG**

**EMG Summary Table**

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<tr>
<td>L. TRICEPS</td>
<td>N</td>
<td>2+</td>
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<td>L. FRONTALIS</td>
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<td>1+</td>
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<tr>
<td>L. TIB ANTERIOR</td>
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</tr>
<tr>
<td>L. GASTROCN (MED)</td>
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<td>2+</td>
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</tbody>
</table>

*Figure 2. Pre-operative EMG of patient 8.*
One year later his neurologic findings had slightly regressed but he was still better compared with the pre-operative period.

**Case 10**
A 44-year-old male was diagnosed with ALS in 2004. Before stem cell therapy his neurologic findings were: tetraplegic; mimic muscles intact. He could not breathe by himself, his swallowing was not good, he could not talk and he was bound to mechanical ventilation (bulbar score 3, Norris scale 17). Three weeks after the stem cell operation his neurologic findings were: muscle strength at upper and lower extremities, 1/5; spontaneous breathing but needed a mechanical ventilator during sleep. Two months later he died because of severe lung infection.

**Case 11**
A 39-year-old female was diagnosed with ALS in 2002. Before stem cell therapy her neurologic findings were: muscle strength of upper and lower extremities, 1/5; she could not walk. Her talking had been getting worse in the last 2 years, and her swallowing was getting worse, especially in the 3 months prior to the study (bulbar score 6, Norris scale 41). Three weeks after the stem cell operation her neurologic findings were: muscle strength of upper extremities 3/5, lower extremities 4/5; he could walk. His talking and swallowing were getting better compared with the pre-operative period and his breathing was very good. He died 1.5 months later because of a myocardial infarction (MI).

**Discussion**
The therapeutic target in ALS has shifted from being neuron-centered to focussing on the interaction between motor neurons and non-neuronal cells. With the lack of effective drug treatments for ALS, and compelling pre-clinical data, stem cell research has highlighted this disease as a candidate for stem cell treatment [3,9]. Blood stem cells are the most suitable candidates for human therapies because of their differentiative potential and easy access. Jlang et al. [12] has described rodent and human BM subpopulations co-purifying with mesenchymal stromal cells (MSC) and called them multipotent adult progenitor cells. These cells differentiated into cells with mesoderm, endoderm and neuroectoderm characteristics in vitro and...
in vivo after transplantation, showing extensive proliferation without loss of differentiation potential, similarly to embryonic stem cells. The transdifferentiation of BM-derived cells into neurons in the central nervous system has been shown in humans. Even better results have been achieved in vitro with human hematopoietic stem cells from umbilical cord, which differentiated sequentially into neuronal stem cells and then astrocytes in a suitable microenvironment [13]. Janson et al. [14] injected peripheral blood-purified CD34+ cells intrathecally in three ALS patients. After 6–12 months, none of the patients reported side-effects but no clinical efficacy was seen. Mazzini et al. [15–17] injected autologous BM-derived cells, after expansion in vitro in seven ALS patients, into surgically exposed spinal cord at T7–T9. A significant slowing down of the linear decline of the forced vital capacity was evident in four patients 36 months after MSC transplantation. These authors concluded that direct injection of autologous expanded MSC into the spinal cord of ALS patients is safe, with no significant acute or late toxicity, and well tolerated [15–17].

In this study, we used BM-derived hematopoietic progenitor stem cells with our ALS patients. We selected patients with bulbar involvement and severe loss of movement. Our strategy was to put the stem cells into the end of the brain stem and beginning of the spinal cord because the blood–brain barrier is intact in ALS, and this region was the most affected part in our patients. We also injected stem cells into different areas in the spinal cord in order to avoid direct injury to the spinal cord. MRI performed six months later did not signify any abnormality at the site of the injection. During the follow-up period, none of the patients was under drug therapy for ALS. After stem cell implantation, nine patients were better after 1 year compared with their pre-operative status, as confirmed by their bulbar scores and Norris scales, and in these patients recovery was also confirmed with ENMG. One patient was stable without any decline or improvement in his status. Two patients had improvement after the stem cell implantation but some degree of decline in their status after 6 months, but their situation after 1 year was still better than the pre-operation period, as confirmed by bulbar scores and Norris scales. Seven patients were better than their pre-operative period without any medication. Three patients had died 1.5, 2 and 9 months after the procedure because of lung infection and MI.

Compared with other studies, our treatment has provided more promising results. We believe this is because injection of stem cells directly into the brain stem and upper spinal cord causes an early improvement, while a long-term response results from the slow and long-term release of stem cells from the storage material. This slow release provides long-term exposure of the brain stem to stem cells. Hence our unique technique shows promising results and opens up a new era in stem cell treatment.

Neuro-inflammation is thought to play a role in ALS pathogenesis [18]. Activated microglia, the inflammatory cells of the central nervous system, are found in areas of neurodegeneration in human post-mortem specimens, and microglial activation occurs in SOD1 mice [19]. A new finding, seen during the operations, is arachnoid thickness, observed in the first patient, when arachnoiditis was suspected. Biopsies were taken from the arachnoid of another eight patients. These specimens were evaluated and lymphocytic arachnoiditis was observed in five patients (Figures 4 and 5). These findings could be important for the etiology of ALS but should be confirmed with further investigations.

These results show that stem cell therapy is a safe and effective treatment for ALS patients. The patients had some degree of decline 1 year after stem cell therapy but they were still better compared with the pre-operative period and without any drug therapy for ALS. Our opinion is that these encouraging results are not only because of implanted exogenous stem cells but also because of activated endogenous stem cells. However, because of the degree of decline in some patients, repeated times of cell

Figure 4. Diffuse lymphocytic infiltration in arachnoidal stroma (HE, × 150).
transplantation may be needed. Further studies with our repeated stem cell therapies will enlighten the therapeutic problems for ALS patients.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References