

Safety and Immunological Effects of Mesenchymal Stem Cell Transplantation in Patients With Multiple Sclerosis and Amyotrophic Lateral Sclerosis

Dimitrios Karussis, MD, PhD; Clementine Karageorgiou, MD; Adi Vaknin-Dembinsky, MD, PhD; Basan Gowda-Kurkalli, PhD; John M. Gomori, MD; Ibrahim Kassis, MSc; Jeff W. M. Bulte, PhD; Panayiota Petrou, MD; Tamir Ben-Hur, MD, PhD; Oded Abramsky, MD, PhD; Shimon Slavin, MD

Objective: To evaluate the feasibility, safety, and immunological effects of intrathecal and intravenous administration of autologous mesenchymal stem cells (MSCs) (also called *mesenchymal stromal cells*) in patients with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS).

Design: A phase 1/2 open-safety clinical trial.

Patients: Fifteen patients with MS (mean [SD] Expanded Disability Status Scale [EDSS] score, 6.7 [1.0]) and 19 with ALS (mean [SD] Amyotrophic Lateral Sclerosis Functional Rating Scale [ALSFRS] score, 20.8 [8.0]) were enrolled.

Intervention: After culture, a mean (SD) of 63.2×10^6 (2.5×10^6) MSCs was injected intrathecally ($n=34$) and intravenously ($n=14$). In 9 cases, MSCs were magnetically labeled with the superparamagnetic iron oxide ferumoxides (Feridex).

Main Outcome Measures: The main outcome measure was the recording of side effects. Follow-up (≤ 25 months) included adverse events evaluation, neurological disability assessment by means of the EDSS, magnetic resonance imaging to exclude unexpected pathologies and track the labeled stem cells, and immunological

tests to assess the short-term immunomodulatory effects of MSC transplantation.

Results: Twenty-one patients had injection-related adverse effects consisting of transient fever, and 15 reported headache. No major adverse effects were reported during follow-up. The mean ALSFRS score remained stable during the first 6 months of observation, whereas the mean (SD) EDSS score improved from 6.7 (1.0) to 5.9 (1.6). Magnetic resonance imaging visualized the MSCs in the occipital horns of the ventricles, indicating the possible migration of ferumoxides-labeled cells in the meninges, subarachnoid space, and spinal cord. Immunological analysis revealed an increase in the proportion of $CD4^+CD25^+$ regulatory T cells, a decrease in the proliferative responses of lymphocytes, and the expression of $CD40^+$, $CD83^+$, $CD86^+$, and HLA-DR on myeloid dendritic cells at 24 hours after MSC transplantation.

Conclusion: Transplantation of MSCs in patients with MS and ALS is a clinically feasible and relatively safe procedure and induces immediate immunomodulatory effects.

Trial Registration: clinicaltrials.gov Identifier: NCT00781872

Arch Neurol. 2010;67(10):1187-1194

MULTIPLE SCLEROSIS (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that leads to cumulative and irreversible CNS damage.¹⁻³ Over time, therapeutic approaches to MS were aimed at suppressing the immune system to control the inflammatory process that causes the demyelination and axonal damage.^{2,3} However, the MS treatments available to date are only partially effective, especially in the progressive phases of the disease.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that selectively affects motor neurons in the brain and spinal cord, leading to bulbar, respiratory, and limb weakness. There is no effective treatment, and the disease usually progresses to death within 2 to 4 years.⁴

Previous efforts using various neuroprotective agents in progressive MS and ALS did not prove successful. The use of multipotential stem cells may provide an alternative solution because stem cells can migrate locally into damaged CNS areas where they have the potential to support

Author Affiliations are listed at the end of this article.

local neurogenesis or myelogenesis through neurotrophic effects, stimulation of resident CNS stem cells, induction of in situ immunomodulation, or, theoretically, even transdifferentiation.

Mesenchymal stem cells (MSCs) (also called *mesenchymal stromal cells*) are bone marrow–derived stem cells that normally generate osteocytes, adipocytes, and chondrocytes.^{5,6} Mesenchymal stem cells have been shown to possess immunomodulating properties, inducing suppression of various immune cell populations.⁷⁻¹⁴ Mesenchymal stem cells cultivated under different culture manipulations (chemical induction or use of growth factors) can give rise to neural-like, glial-like, and astrocytic-like cells in vitro.¹⁵⁻¹⁹ In rats with an induced focal demyelinated lesion of the spinal cord, intravenous or intracerebral injection of MSCs resulted in remyelination.^{20,21} In the animal model of MS, experimental autoimmune encephalomyelitis, intravenous injection of MSCs into C57BL/6J mice was shown to downregulate the clinical severity of the disease with a parallel suppression of CNS inflammation through induction of T-cell anergy and decrease of demyelination.²²⁻²⁵ Mesenchymal stem cells migrated into the CNS, where they promoted brain-derived neurotrophic factor production and induced proliferation of a limited number of oligodendrocyte progenitors. In our previous study,²⁵ intraventricularly injected MSCs migrated to the white matter lesions in correlation with the degree of inflammation and induced neuroprotection, with preservation of the axons.²⁵ Similar beneficial clinical effects of MSC transplantation were described in models of stroke and trauma.²⁶

Clinical trials have revealed the feasibility and safety of the clinical use of MSCs (for review, see Giordano et al²⁷) and have provided some evidence of efficacy in various medical conditions.²⁸⁻³⁴

On the basis of the preclinical experience and the cumulative data from clinical studies,²⁸⁻³⁹ we initiated an exploratory trial with autologous bone marrow–derived MSCs in 34 patients with intractable MS or progressive ALS. We combined intrathecal and intravenous administration to maximize the potential therapeutic benefit by accessing the CNS through the cerebrospinal fluid and the systemic circulation. In 9 patients, MSCs were labeled with the superparamagnetic iron oxide magnetic resonance imaging (MRI) contrast agent ferumoxides (Feridex)⁴⁰⁻⁴³ to track cell migration after local grafting.

METHODS

DESIGN OF TRIAL AND PATIENT POPULATION

This study, designed as a phase 1/2 open-safety clinical trial, was approved by the ethics committees of the Gennimatas General Hospital and Hadassah Hebrew University Hospital and registered in the National Institutes of Health database. We included 15 consenting patients with MS (7 men and 8 women; mean age, 35.3 [8.6] years) with a mean disease duration of 10.7 (2.9) (range, 5-15) years and baseline Expanded Disability Status Scale (EDSS) score of 6.7 (1.0) (range, 4.0-8.0), and we also included 19 patients with ALS (10 men and 9 women; mean age, 53.0 [11.2] years) with a disease duration of 34.3 (20.6) (range, 6-84) months and a mean baseline Amyotro-

phic Lateral Sclerosis Functional Rating Scale (ALSFRS) score of 20.8 (8.0). (Unless otherwise indicated, data are given as mean (SD).) All patients signed an informed consent approved by the institutional review boards of both centers.

MS Inclusion Criteria

Consenting patients fulfilled the following 4 inclusion criteria for this study: (1) the clinical criteria of Poser et al⁴⁴ for definite MS; (2) men and nonpregnant women aged 25 to 65 years; (3) duration of disease longer than 5 years; and (4) failure to respond to the currently available and registered agents for MS (ie, interferons, glatiramer acetate [Copaxone], and immunosuppressors), as manifested by an increase of at least 1 degree in the EDSS score during the past year or the appearance of at least 2 major MS relapses during the same period. We excluded MS patients (1) who were treated with cytotoxic medications (ie, cyclophosphamide, mitoxantrone, and azathioprine) during the 3 months before the trial; (2) who had significant cardiac, renal, or hepatic failure or any other disease that may interfere with the ability to interpret the results of the study; (3) who had an active infection; and (4) who showed severe cognitive decline or were unable to understand and sign the informed consent.

ALS Inclusion Criteria

Consenting patients fulfilled the following 3 inclusion criteria for this study: (1) meeting the El Escorial criteria for definite ALS⁴⁵; (2) being men or nonpregnant women aged 25 to 65 years; and (3) having a progressive course, with evidence of deterioration of at least 5 degrees in the ALSFRS scale of disease severity during the year preceding inclusion in the trial. We excluded ALS patients with (1) high protein levels or lymphocytosis in the cerebrospinal fluid; (2) positive test results for anti-GM1 antibodies; (3) significant conduction blocks or slow conduction velocities (a reduction of >30%) in nerve conduction studies; (4) significant cardiac, renal, or hepatic failure or any other disease that may interfere with the ability to interpret the results of the study; (5) an active infection; and (6) cognitive decline or the inability to understand and sign the informed consent.

TREATMENT PROTOCOL

Bone Marrow Aspiration

Bone marrow aspiration was performed under short general anesthesia with puncture from the posterior superior iliac crest while the patient was lying in a left or a right lateral position. Approximately 200 mL of bone marrow inocula was obtained from each patient.

MSC Preparation and Culture

A culture of purified MSCs was prepared under aseptic conditions (positively pressurized clean rooms) using filtered sterilized Dulbecco modified Eagle medium with low glucose levels (Qiagen, Valencia, California) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin solution (all from Biological Industries, Kibbutz Beit-Haemek, Israel).

Mesenchymal cells were cultured for 40 to 60 days, until they reached confluency, and were then harvested and cryopreserved in 10% dimethyl sulfoxide–containing medium in liquid nitrogen (–196°C). At 2 weeks, a sample was taken for sterility testing and quality control. After sterility was confirmed,

the MSCs were transferred to the laboratory on dry ice, thawed in a 37°C water bath, and washed twice with normal saline solution to remove any residual dimethyl sulfoxide. The cells were then resuspended in normal saline at a concentration of $10 \times 10^6/\text{mL}$ to $15 \times 10^6/\text{mL}$. Two-thirds of the total number of cells (usually 60×10^6 to 100×10^6) were injected intrathecally, and one-third was injected intravenously. A sample of the cells to be injected was tested by fluorescence-activated cell sorter (FACS) analysis; cells consistently (>98%) expressed the surface markers characteristic of MSCs (CD29, CD73, CD90, CD105, and CD166) and were negative for CD34, CD45, and CD14.

Treatment Protocol

All patients received an intrathecal injection via a standard lumbar puncture. Patients with MS received a mean of 63.2×10^6 (2.5×10^6) cells; patients with ALS received 54.7×10^6 (17.4×10^6) cells in 2 mL of normal saline solution. Fourteen patients (5 with MS and 9 with ALS) also received intravenous MSCs (mean, 24.5×10^6 [2.5×10^6] for MS and 23.4×10^6 [6.0×10^6] for ALS, in 2 mL of normal saline solution). Nine patients received MSCs incubated with superparamagnetic iron oxide (ferumoxides) to detect their trafficking and migration by MRI. To this end, ferumoxides was complexed with the cationic polymer poly-L-lysine, and the cells were incubated for 24 to 48 hours as described in detail elsewhere.⁴⁶

MAGNETIC RESONANCE IMAGING

We performed MRI on all patients within 4 to 48 hours after MSC infusion and again after 1 month and 3 to 6 months. The MRI examinations were used to exclude unexpected pathologies and also to track the CNS homing of injected MSCs in patients whose cells were labeled with ferumoxides. All MRIs were performed at 1.5 T with the exception of one performed at 3 T. Brain imaging was performed using standard T1-, T2-, diffusion-, and postgadolinium T1-weighted sequences. Whole spine imaging was performed using standard T1-, T2-, and postgadolinium T1-weighted sequences.

IMMUNOLOGICAL EVALUATION

Immunological analysis of lymphocyte subsets and cytokine production was performed in 12 patients (5 with ALS and 7 with MS, all of them undergoing intrathecal and intravenous transplantation) at baseline and 4 and 24 hours after MSC administration. The tests are described in the following paragraphs.

FACS Analysis of Lymphocyte Subsets

Peripheral blood monocytes were centrifuged using gradient cell separation medium (Histopaque 1077; Sigma-Aldrich Corp, St Louis, Missouri) and stained for flow cytometric analysis with anti-CD4 phycoerythrin (PE) and CD25-fluorescein isothiocyanate conjugate (FITC) (BD Biosciences, Mountain View, California). The isolated peripheral blood monocytes were also stained for the following myeloid dendritic markers: lineage cocktail FITC (BD Biosciences), CD11c antigen-presenting cells (Biotest Pharmaceuticals, Boca Raton, Florida), CD86 PE, CD83 PE, CD40 PE, and HLA-DR PE (eBioscience Inc, San Diego, California). The data were analyzed with the aid of a flow cytometer (Beckman Coulter, Brea, California).

Table 1. Adverse Events in Patients With MS and With ALS After MSC Transplantation

Adverse Event	No. of Patients		Total No. (%) (N=34)
	ALS Group (n=19)	MS Group (n=15)	
Fever	11	10	21 (61.8)
Headache	5	10	15 (44.1)
Meningism	0	1	1 (2.9)
Rigidity	0	2	2 (5.9)
Leg pain	2	1	3 (8.8)
Dyspnea	1	0	1 (2.9)
Confusion	0	1	1 (2.9)
Neck pain	0	1	1 (2.9)
Difficulty walking/standing	0	4	4 (11.8)

Abbreviations: ALS, amyotrophic lateral sclerosis; MS, multiple sclerosis; MSC, mesenchymal stem cell.

Lymphocyte Proliferation in Response to Phytohemagglutinin

The assay was performed in 96-well, flat-bottom plates (Nunc plates; Danyl Biotech, Rehovot, Israel). Lymphocytes were isolated from whole blood by centrifugation using the gradient cell separation medium and seeded at $25 \times 10^5/\text{well}$ in a mixture of RPMI (Roswell Park Memorial Institute) tissue culture medium, 10% fetal calf serum, 1 mM glutamine, and penicillin-streptomycin (Biological Industries) and stimulated with the lectin phytohemagglutinin, 1 $\mu\text{g}/\text{mL}$ (Sigma-Aldrich Corp). Cultures were incubated for 48 hours in a humidified atmosphere of 5% carbon dioxide at 37°C, and then proliferation was assayed using 1 $\mu\text{Ci}/\text{well}$ of tritiated thymidine (Amersham, Aylesbury, England) uptake. After 18 hours of incubation with tritiated thymidine, the cells were frozen in -20°C and then harvested on fiberglass filters using a cell harvester (Skatron Instruments, Lier, Norway); radioactivity was measured by a standard scintillation technique. The stimulation index was calculated as the ratio of the activated to the nonactivated cells.

RESULTS

SAFETY AND CLINICAL EFFECTS OF MSC TRANSPLANTATION

Safety

Of the 34 patients, 21 had a mild self-limited febrile reaction (temperature, $\leq 37.5^\circ\text{C}$) that lasted for 8 to 24 hours after MSC injection (**Table 1**). Headaches, which lasted for up to 7 days, were reported in 15 patients and were mainly related to the lumbar puncture. Meningeal irritation and aseptic meningitis was observed in 1 patient, and a second lumbar puncture was performed in that case to rule out the possibility of infection. Aseptic meningitis was diagnosed and was most likely caused by residual dimethyl sulfoxide in the culture medium owing to insufficient washing of the cells (Table 1). The adverse effects profile did not differ significantly between the MS and ALS groups. No major adverse effects were reported in any of the patients during a follow-up of up to 25 months.

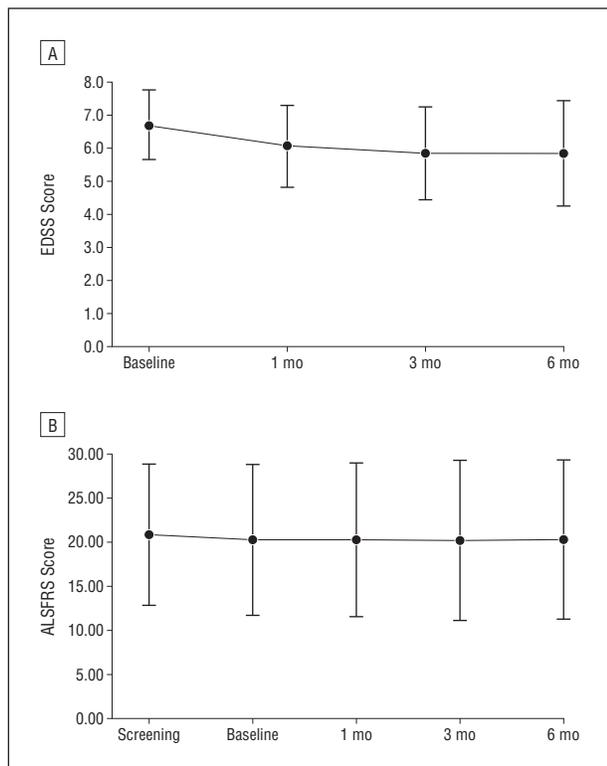


Figure 1. Clinical follow-up of patients with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) after transplantation of mesenchymal stem cells (MSCs). A, The Expanded Disability Status Scale (EDSS) score in patients with MS was significantly reduced at 1 ($P < .001$), 3 ($P < .001$), and 6 ($P = .001$) months, compared with baseline (2-tailed paired t test). B, Changes in the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) score were not statistically significant.

Clinical Effects

Figure 1 shows the follow-up of the mean EDSS and ALSFRS scores at baseline and at 1, 3, and 6 months after MSC transplantation. In patients with MS, the mean EDSS score declined gradually (indicating functional improvement) from 6.7 (1.0) before the treatment to 6.1 (1.2) at 1 month, 5.9 (1.4) at 3 months, and 5.9 (1.6) at 6 months after MSC injection ($P < .001$, $P < .001$, and $P = .001$, respectively, 2-tailed paired t test) (Figure 1A). Although the follow-up of the mean EDSS scores in the whole group is not the optimal way to assess treatment efficacy in small groups, it may provide some indication of positive effects and, most important in such a phase 1/2 study, confirm the lack of any deleterious clinical effects. More specifically, at the end of the 6 months of follow-up, the EDSS score remained unchanged in 4 patients and was reduced by 0.5 degree in 5. It improved by 1.0 degree in 1 patient, by 1.5 degrees in 3, by 2 degrees in 1, and by 2.5 degrees in 1. The EDSS score did not deteriorate in any of the patients.

In the patients with ALS, the mean ALSFRS score deteriorated slightly during the 2 months between the screening visit and the day of MSC injection but thereafter remained stable during the 6-month follow-up (20.1-20.5, with no statistically significant difference between time points) (Figure 1B).

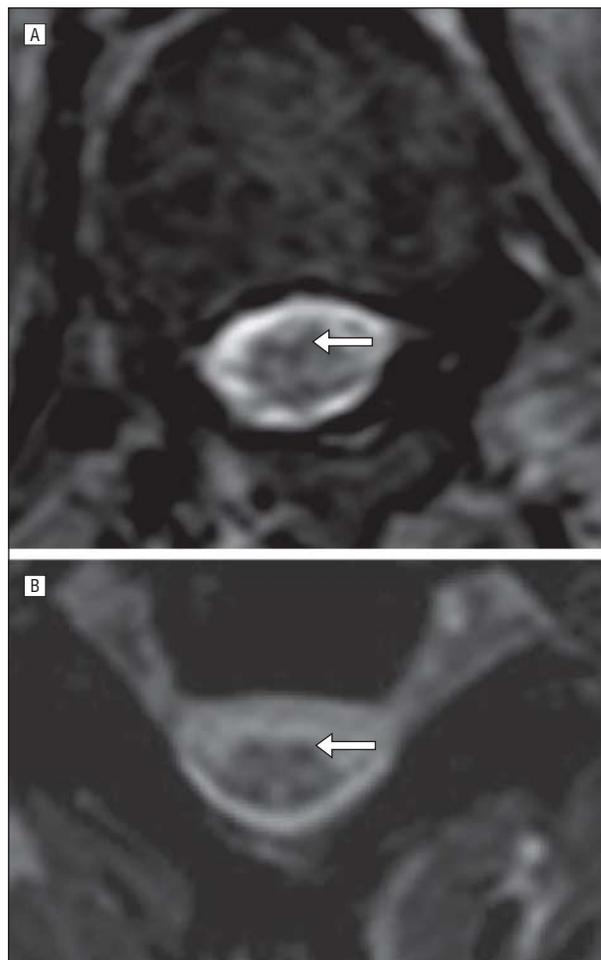


Figure 2. Magnetic resonance imaging after injection of ferumoxides-labeled mesenchymal stem cells. A, An axial T2-weighted gradient echo scan through the inferior thoracic cord shows a hypointense pial signal coating the cord similar to that of superficial siderosis, characteristic of ferumoxides (Feridex)-labeled cells. B, Axial T2-weighted gradient echo scan through the cervical cord shows hypointensity of the dorsal roots and their entry zone and a similar hypointensity of the ventral root entry zones, suggesting the presence of ferumoxides-labeled cells.

NEURORADIOLOGICAL EFFECTS OF MSC TRANSPLANTATION

Magnetic resonance imaging (1.5 T) of the brain and whole spine during the 6-month follow-up did not reveal any significant unexpected pathology. In the MS group, no new or gadolinium-enhancing lesions were observed in the brain for up to 6 months after MSC treatment. In the 9 patients in whom the MSCs were labeled with ferumoxides, MRI of the brain and whole spine was performed at 24 to 48 hours and at 1 to 3 months after injection of MSCs. Hypointense signals in T2-weighted images, indicating the presence of ferumoxides-positive cells, were detected in the meninges of the spinal cord and nerve roots and in the spinal cord parenchyma (Figure 2). In 1 patient who received MSCs without ferumoxides labeling, a 3-T brain MRI performed 18 hours after transplantation (Figure 3) showed dependent layering of the intrathecally delivered cells in the occipital horns, suggesting dissemination of MSCs from the injection site to the ventricles of the CNS.

IMMUNOLOGICAL EFFECTS OF MSC TRANSPLANTATION

To evaluate the *in vivo* immunoregulatory effects of MSC transplantation, peripheral blood monocytes were obtained from 12 patients (5 with ALS and 7 with MS), and the changes in the expression of cell surface markers and the lymphocyte proliferative responses on stimulation with phytohemagglutinin were tested before and at 4 and 24 hours after MSC administration. Analysis of the data in all 12 patients together (as a single group) using a 2-tailed paired *t* test showed a 72% increase in the proportion of CD4⁺CD25⁺ regulatory T cells (from 8.3% [6.4%] to 14.2% [7.5%]; *P*=.02) and a 30% to 60% reduction of CD86⁺ (from 82.6% [20.5%] to 58.8% [16.3%]; *P*=.02), CD83⁺ (from 26.6% [8.4%] to 12.3% [13.2%]; *P*=.02), and HLA-DR⁺ (from 92.1% [5.2%] to 74.6% [12.1%]; *P*=.004) myeloid dendritic cells and a similar reduction in the number of activated CD40⁺ cells (from 22.9 [5.3] to 10.7 [14.0]; *P*=.04) 24 hours after MSC infusion (**Figure 4A** and **Table 2**). These changes were similar in the MS and ALS groups when analyzed separately (Table 2). In addition, after stimulation of lymphocytes with the phytohemagglutinin, there was a 63% decrease in the proliferative cell response (stimulation index at baseline, 26.6 [4.1]; 24 hours later: 9.6 [4.8]; *P*=.001, 2-tailed paired *t* test) (Figure 4B). Although it is difficult to estimate the clinical relevance of these immunological effects, changes of that magnitude are stronger than those induced by the conventional immunomodulatory medications and indicate a down-regulation of activated lymphocytes and antigen-presenting cells and the proliferative ability of effector cells after MSC transplantation.

COMMENT

Our phase 1/2 pilot clinical trial using combined intrathecal and intravenous injection of bone marrow-derived autologous MSCs in 34 patients with MS and ALS was aimed at exploring the feasibility and safety of this type of cell therapy. The 6 to 25 months of follow-up did not reveal any significant immediate or late adverse effects and indicated clinical stabilization or improvement in some patients. Magnetic resonance imaging indicated possible dissemination of the MSCs from the lumbar site of inoculation to the occipital horns, meninges, spinal roots, and spinal cord parenchyma (Figures 2 and 3). Immunological analysis of lymphocyte subsets and cytokine production, performed in 12 patients, demonstrated the immediate *in vivo* immunomodulating effects of MSCs, starting as early as 4 hours after MSC transplantation and including an increase in CD4⁺CD25⁺ regulatory cells and a reduction in the proportion of activated dendritic cells and lymphocytes and of lymphocyte proliferation (Figure 4).

One of the possible approaches to enhancing neuroprotective mechanisms and inducing neuroregeneration in progressive MS and ALS may involve the use of adult or nonembryonic stem cells, which are more differentiated than embryonic stem cells and can be harvested from various tissues. Bone marrow MSCs mainly support the

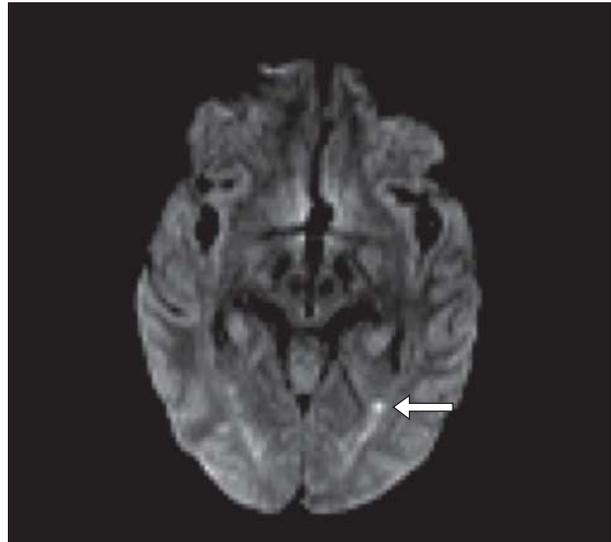


Figure 3. A 3-T diffusion-weighted axial magnetic resonance imaging scan of the brain shows hyperintense signals in the occipital horns of the brain ventricles, indicating the presence of dependent transplanted cells that were not magnetically labeled.

processes of hematopoiesis and hematopoietic stem cell engraftment but can also give rise to cells of mesodermal origin such as osteoblasts, adipocytes, and chondrocytes. Recent studies have described the following additional properties of MSCs: (1) a debatable ability to transdifferentiate into cells of endodermal and ectodermal origin,^{6,47,48} including possible neural transdifferentiation,^{15,17,19} and (2) systemic (peripheral) and local (in the CNS) immunomodulatory effects.^{8,9,49-51}

The use of bone marrow-derived stem cells offers several practical advantages: (1) MSCs can be obtained readily and safely from adult bone marrow, even from patients with advanced disease; (2) MSCs, which are normally present in small concentrations in the bone marrow compartment, can be enriched and greatly expanded by *in vitro* culturing; (3) autologous MSCs can be administered safely without the need for immunosuppressive treatment to prevent rejection; and (4) adult MSCs were shown to be less prone to genetic abnormalities and malignant transformation during multiple passages *in vitro*, thus implying a low risk for induction of treatment-related malignant neoplasms.⁵²⁻⁵⁵

The preclinical studies,²⁸⁻³⁹ together with the cumulative data from ongoing clinical trials with MSCs in various clinical conditions (reviewed by Giordano et al²⁷), provided the scientific basis for our trial. The only available data on the use of MSCs in neurological conditions include a small study⁵⁶ in 7 patients with ALS and a trial from Iran⁵⁷ that did not report any significant adverse events. Two additional, recently published studies, a phase 1 trial in patients with ALS (with intraspinal injection of MSCs)⁵⁸ and a small pilot study with 3 patients with MS that used intravenous administration of adipose tissue MSCs,⁵⁹ also support the safety of the use of MSCs.

Our main finding was the feasibility and acceptable safety profile of transplantation of autologous stem cells from the bone marrow in patients with MS and ALS. None of our patients experienced significant adverse effects dur-

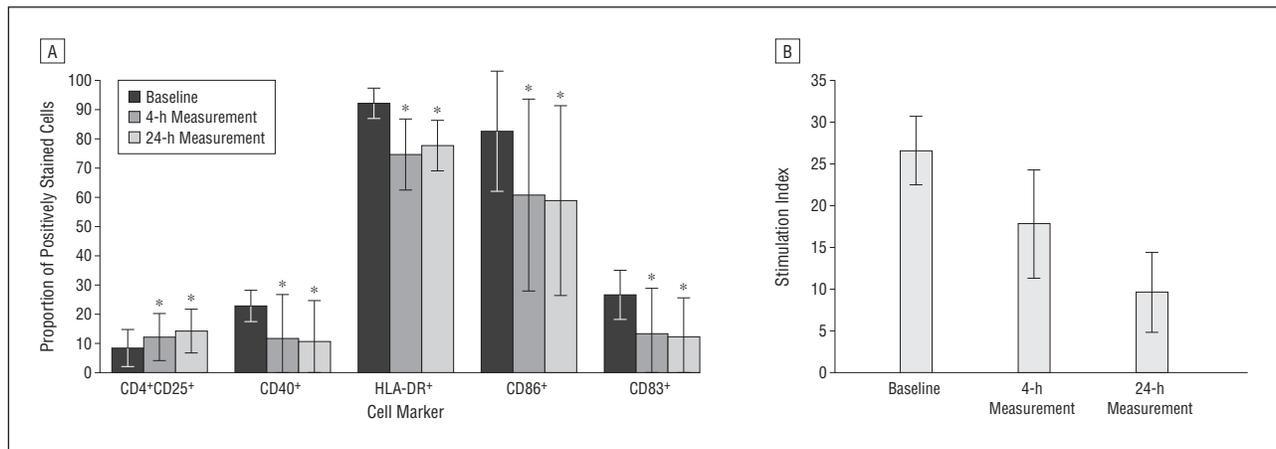


Figure 4. Immunological effects in patients with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) injected intravenously and intrathecally with mesenchymal stem cells (MSCs). Peripheral blood monocytes were obtained from 12 patients (7 with MS and 5 with ALS, combined as a single group) at baseline and at 4 and 24 hours after autologous MSC transplantation. A, Mean (SD) changes in the proportions of CD4⁺CD25⁺ and CD40⁺ lymphocytes and of CD83⁺, CD86⁺, and HLA-DR⁺ myeloid dendritic cells (fluorescence-activated cell sorter analysis), at 4 and 24 hours after MSC transplantation. *Statistically significant changes ($P < .05$) compared with baseline (2-tailed paired t test). B, Changes in lymphocytic proliferation on stimulation with phytohemagglutinin after MSC transplantation (tested by means of tritiated thymidine uptake of peripheral blood lymphocytes obtained from MSC-treated patients with ALS and with MS that were then stimulated with phytohemagglutinin), at 4 and 24 hours after MSC transplantation. The differences were statistically significant ($P = .001$) compared with baseline (2-tailed paired t test).

Table 2. Immunological Effects in Patients With MS and With ALS Undergoing MSC Transplantation Intravenously and Intrathecally^a

Lymphocyte Subpopulation	Mean (SD) Proportions of Lymphocytes		
	Baseline	4 h After MSC Transplantation	24 h After MSC Transplantation
Patients with MS (n=7)			
CD4 ⁺ CD25 ⁺	8.4 (6.3)	10.0 (4.5)	12.0 (3.7)
CD86 ⁺	93.6 (4.7)	78.7 (14.3)	74.3 (11.0)
CD40 ⁺	26.0 (4.0)	13.4 (7.15)	11.5 (8.4)
HLA-DR ⁺	95.7 (3.8)	81.3 (9.3)	81.0 (8.5)
CD83 ⁺	32.4 (4.9)	20.1 (3.4)	19.2 (6.2)
Patients with ALS (n=5)			
CD4 ⁺ CD25 ⁺	8.3 (2.6)	13.7 (7.2)	16.2 (5.3)
CD86 ⁺	74.0 (23.5)	50.0 (18.4)	48.7 (28.3)
CD40 ⁺	12.9 (4.1)	5.2 (6.0)	7.0 (6.6)
HLA-DR ⁺	88.6 (4.2)	68.0 (12.2)	74.3 (9.1)
CD83 ⁺	22.4 (2.1)	18.1 (7.1)	17.2 (3.2)

Abbreviations: ALS, amyotrophic lateral sclerosis; MS, multiple sclerosis; MSC, mesenchymal stem cell.

^aValues represent the proportions of positively stained cells detected by fluorescence-activated cell sorter analysis. For all measurements at 4 and 24 hours after MSC transplantation, changes were significant compared with baseline ($P < .05$, 2-tailed t test).

ing the 6- to 25-month observation. In 20 patients, follow-up MRI 1 year after transplantation did not reveal any unexpected pathology or significant new activity of the disease.

Several clinical trials in nonneurological diseases²⁸⁻³⁹ have indicated that intravenous administration of MSCs is a safe procedure. Our study additionally shows an acceptable short-term safety profile of the intrathecal route of administration of stem cells at doses of up to 70 million cells per injection per patient. The intrathecal approach for cell-based therapies in neurological diseases

such as MS and ALS, in which the areas of tissue damage are widespread throughout the neuroaxis, may increase the possibility of migration of the injected cells to the proximity of the CNS lesions. The injected cells may circulate with the flow of the cerebrospinal fluid and have a better chance of reaching the affected CNS areas. Our animal studies showed that this route of administration could induce superior neurotrophic and neuroprotective effects.²⁵ However, the optimal route of stem cell administration in general—and particularly MSC administration—in patients with neurological diseases remains debatable. Other investigators have claimed that intravenous injection may be sufficient and equally effective (at least in the case of MS) because MSCs exert peripheral immunomodulating effects and may also migrate through the blood to the damaged areas of the CNS after receiving inflammatory signals.²³⁻²⁵ A possible drawback of the intravenous administration of MSCs is that most of the cells injected into the blood will home to the lungs, lymph nodes, and other tissues, greatly reducing the number of cells available to migrate to the CNS. Moreover, intrathecal delivery of cells may focus their possible immunomodulatory and trophic effects directly on the CNS, without producing systemic adverse effects.

The initial findings of our trial support the possibility of migration of MSCs from their site of injection (lumbar area of the cerebrospinal fluid) to the brain ventricles and spinal cord parenchyma. Despite the absence of definite proof, the hypointense signals in the meninges and the spinal cord parenchyma, shown in our MRI studies (Figure 2), may indicate the presence of superparamagnetic particles (ferumoxides-labeled MSCs) in these CNS areas. However, the hypointense areas could also be related to the presence of macrophages that phagocytized the iron oxide magnetic resonance contrast agent and migrated to the inflammatory MS lesions.

Our data also demonstrate and confirm, to our knowledge for the first time in human neurological diseases,

the in vivo systemic immunomodulatory effects of MSCs previously described in animal studies.²⁵ The finding of early clinical stabilization or improvement in some of the patients could be related to these immunomodulating effects. The possibility of neuroprotection and neuroregeneration through transdifferentiation of MSCs into cells of the neuronal or glial lineage, although theoretically viable, has yet to be proved by neuroimaging studies. Further controlled trials are warranted to evaluate the long-term safety and the potential clinical efficacy of MSC transplantation. According to recent consensus papers,^{60,61} intravenous injection of MSCs (at a suggested dose of 10⁶/kg, which has been shown to be optimal for effective immunomodulation) seems to be the most feasible approach in designing future efficacy trials in patients with active MS.

Accepted for Publication: May 25, 2010.

Author Affiliations: Department of Neurology, Laboratory of Neuroimmunology and Agnes Ginges Center for Neurogenetics and Multiple Sclerosis Center (Drs Karussis, Vaknin-Dembinsky, Petrou, Ben-Hur, and Abramsky and Mr Kassis), Department of Bone Marrow Transplantation (Drs Gowda-Kurkalli and Slavin), and Unit of Neuroradiology, Department of Radiology (Dr Gomori), Hadassah Hebrew University Hospital, Jerusalem, Israel; Department of Neurology, Gennimatas General Hospital, Athens, Greece (Dr Karageorgiou); and Russell H. Morgan Department of Radiology and Radiological Science, Department of Biomedical Engineering, and Department of Chemical and Biomolecular Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland (Dr Bulte).

Correspondence: Dimitrios Karussis, MD, PhD, Agnes Ginges Center for Neurogenetics and Multiple Sclerosis Center and Department of Neurology, Hadassah-Hebrew University Hospital, Ein Karem, Jerusalem IL-91120, Israel (karus@cc.huji.ac.il).

Author Contributions: *Study concept and design:* Karussis, Vaknin-Dembinsky, Gomori, Bulte, Petrou, Ben-Hur, Abramsky, and Slavin. *Acquisition of data:* Karussis, Karageorgiou, Vaknin-Dembinsky, Gowda-Kurkalli, Gomori, Kassis, Petrou, Ben-Hur, Abramsky, and Slavin. *Analysis and interpretation of data:* Karussis, Vaknin-Dembinsky, Gomori, Kassis, Bulte, Petrou, Ben-Hur, Abramsky, and Slavin. *Drafting of the manuscript:* Karussis, Karageorgiou, Vaknin-Dembinsky, Gowda-Kurkalli, Gomori, Kassis, Bulte, Petrou, Ben-Hur, Abramsky, and Slavin. *Critical revision of the manuscript for important intellectual content:* Karussis, Vaknin-Dembinsky, Gomori, Bulte, Petrou, Ben-Hur, Abramsky, and Slavin. *Statistical analysis:* Karussis and Kassis. *Obtained funding:* Karussis and Bulte. *Administrative, technical, and material support:* Karussis, Karageorgiou, Vaknin-Dembinsky, Gowda-Kurkalli, Gomori, Kassis, Bulte, Petrou, Ben-Hur, Abramsky, and Slavin. *Study supervision:* Karussis, Gowda-Kurkalli, Gomori, Petrou, and Slavin.

Financial Disclosure: None reported.

Funding/Support: This work was supported by the ECTRIMS Fellowship grant 2009-2010. Part of this study was funded by NMSS RG3630 and TEDCO MD Stem Cell Fund ESC 06-29-01.

- Karussis D, Grigoriadis S, Polyzoidou E, Grigoriadis N, Slavin S, Abramsky O. Neuroprotection in multiple sclerosis. *Clin Neurol Neurosurg.* 2006;108(3):250-254.
- Steinman L. Multiple sclerosis: a two-stage disease. *Nat Immunol.* 2001;2(9):762-764.
- Einstein O, Grigoriadis N, Mizrachi-Kol R, et al. Transplanted neural precursor cells reduce brain inflammation to attenuate chronic experimental autoimmune encephalomyelitis. *Exp Neurol.* 2006;198(2):275-284.
- Sabatelli M, Madia F, Conte A, et al. Natural history of young-adult amyotrophic lateral sclerosis. *Neurology.* 2008;71(12):876-881.
- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* 1997;276(5309):71-74.
- Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284(5411):143-147.
- Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation.* 2003;75(3):389-397.
- Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood.* 2006;107(4):1484-1490.
- Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevasis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. *Stem Cells.* 2006;24(1):74-85.
- Rasmuson I, Ringdén O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. *Transplantation.* 2003;76(8):1208-1213.
- Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood.* 2004;103(12):4619-4621.
- Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood.* 2005;105(7):2821-2827.
- Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 2002;99(10):3838-3843.
- Deng W, Han Q, Liao L, You S, Deng H, Zhao RC. Effects of allogeneic bone marrow-derived mesenchymal stem cells on T and B lymphocytes from BXSB mice. *DNA Cell Biol.* 2005;24(7):458-463.
- Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res.* 2000;61(4):364-370.
- Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. *FASEB J.* 2004;18(9):980-982.
- Sanchez-Ramos J, Song S, Cardozo-Pelaez F, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol.* 2000;164(2):247-256.
- Lu P, Blesch A, Tuszynski MH. Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? *J Neurosci Res.* 2004;77(2):174-191.
- Bossolasco P, Cova L, Calzarossa C, et al. Neuro-glial differentiation of human bone marrow stem cells in vitro. *Exp Neurol.* 2005;193(2):312-325.
- Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. *Glia.* 2002;39(3):229-236.
- Inoue M, Honmou O, Oka S, Houkin K, Hashi K, Kocsis JD. Comparative analysis of remyelinating potential of focal and intravenous administration of autologous bone marrow cells into the rat demyelinated spinal cord. *Glia.* 2003;44(2):111-118.
- Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood.* 2005;106(5):1755-1761.
- Zhang J, Li Y, Chen J, et al. Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice. *Exp Neurol.* 2005;195(1):16-26.
- Zhang J, Li Y, Lu M, et al. Bone marrow stromal cells reduce axonal loss in experimental autoimmune encephalomyelitis mice. *J Neurosci Res.* 2006;84(3):587-595.
- Kassis I, Grigoriadis N, Gowda-Kurkalli B, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Arch Neurol.* 2008;65(6):753-761.
- Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci.* 2001;189(1-2):49-57.

27. Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol.* 2007;211(1):27-35.
28. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med.* 1999;5(3):309-313.
29. Horwitz EM, Prockop DJ, Gordon PL, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood.* 2001;97(5):1227-1231.
30. Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet.* 2004;364(9429):141-148.
31. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet.* 2003;361(9351):45-46.
32. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation.* 2003;107(18):2294-2302.
33. Perin EC, Dohmann HF, Borojevic R, et al. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation.* 2004;110(11)(suppl 1):II213-II218.
34. Koç ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant.* 2002;30(4):215-222.
35. Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation.* 2002;106(24):3009-3017.
36. Chen SL, Fang WW, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol.* 2004;94(1):92-95.
37. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors [published correction appears in *Science.* 1998;281(5379):923]. *Science.* 1998;279(5356):1528-1530.
38. Katritsis DG, Sotiropoulou PA, Karvouni E, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv.* 2005;65(3):321-329.
39. Koç ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol.* 2000;18(2):307-316.
40. Bulte JW, Kraitchman DL. Iron oxide MR contrast agents for molecular and cellular imaging. *NMR Biomed.* 2004;17(7):484-499.
41. de Vries IJ, Lesterhuis WJ, Barentsz JO, et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nat Biotechnol.* 2005;23(11):1407-1413.
42. Toso C, Vallee JP, Morel P, et al. Clinical magnetic resonance imaging of pancreatic islet grafts after iron nanoparticle labeling. *Am J Transplant.* 2008;8(3):701-706.
43. Zhu J, Wu X, Zhang HL. Adult neural stem cell therapy: expansion in vitro, tracking in vivo and clinical transplantation. *Curr Drug Targets.* 2005;6(1):97-110.
44. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol.* 1983;13(3):227-231.
45. Brooks BR; Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases; El Escorial "Clinical Limits of Amyotrophic Lateral Sclerosis" Workshop Contributors. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. *J Neurol Sci.* 1994;124(suppl):96-107.
46. Bulte JW, Arbab AS, Douglas T, Frank JA. Preparation of magnetically labeled cells for cell tracking by magnetic resonance imaging. *Methods Enzymol.* 2004;386:275-299.
47. Oswald J, Boxberger S, Jørgensen B, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells.* 2004;22(3):377-384.
48. Taléns-Visconti R, Bonora A, Jover R, et al. Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. *World J Gastroenterol.* 2006;12(36):5834-5845.
49. Krampfer M, Glennie S, Dyson J, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood.* 2003;101(9):3722-3729.
50. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol.* 2003;31(10):890-896.
51. Uccelli A, Zappia E, Benvenuto F, Frassoni F, Mancardi G. Stem cells in inflammatory demyelinating disorders: a dual role for immunosuppression and neuroprotection. *Expert Opin Biol Ther.* 2006;6(1):17-22.
52. Izadpanah R, Trygg C, Patel B, et al. Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. *J Cell Biochem.* 2006;99(5):1285-1297.
53. Draper JS, Moore HD, Ruban LN, Gokhale PJ, Andrews PW. Culture and characterization of human embryonic stem cells. *Stem Cells Dev.* 2004;13(4):325-336.
54. Lee K, Majumdar MK, Buyaner D, Hendricks JK, Pittenger MF, Mosca JD. Human mesenchymal stem cells maintain transgene expression during expansion and differentiation. *Mol Ther.* 2001;3(6):857-866.
55. Asano T, Sasaki K, Kitano Y, Terao K, Hanazono Y. In vivo tumor formation from primate embryonic stem cells. *Methods Mol Biol.* 2006;329:459-467.
56. Mazzini L, Mareschi K, Ferrero I, et al. Autologous mesenchymal stem cells: clinical applications in amyotrophic lateral sclerosis. *Neurol Res.* 2006;28(5):523-526.
57. Mohyeddin Bonab M, Yazdanbakhsh S, Lotfi J, et al. Does mesenchymal stem cell therapy help multiple sclerosis patients? report of a pilot study. *Iran J Immunol.* 2007;4(1):50-57.
58. Mazzini L, Ferrero I, Luparello V, et al. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: a phase I clinical trial. *Exp Neurol.* 2010;223(1):229-237.
59. Riordan NH, Ichim TE, Min WP, et al. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *J Transl Med.* April 2009;7:29.
60. Martino G, Franklin RJ, Van Evercooren AB, Kerr DA; Stem Cells in Multiple Sclerosis (STEMS) Consensus Group. Stem cell transplantation in multiple sclerosis: current status and future prospects. *Nat Rev Neurol.* 2010;6(5):247-255.
61. Freedman MS, Bar-Or A, Atkins HL, et al; MSCT Study Group. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. *Mult Scler.* 2010;16(4):503-510.

Announcement

Calendar of Events: A New Web Feature. On the new Calendar of Events site, available at <http://pubs.ama-assn.org/cgi/calendarcontent> and linked off the home page of the *Archives of Neurology*, individuals can now submit meetings to be listed. Just go to <http://pubs.ama-assn.org/cgi/cal-submit/> (also linked off the Calendar of Events home page). The meetings are reviewed internally for suitability prior to posting. This feature also includes a search function that allows searching by journal as well as by date and/or location. Meetings that have already taken place are removed automatically.