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# Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans

Letizia Mazzini,[1] Franca Fagioli,[2] Riccardo Boccaletti,[3] Katia Mareschi,[2] Giuseppe Oliveri,[3] Carlo Olivieri,[4] Ilaria Pastore,[6] Roberto Marasso[5] and Enrico Madon[2]

Departments of [1]Neurology, [2]Department of Pediatric and Oncohaematology, University of Torino, Torino, Italy, [3]Neurosurgery, [4]Intensive Care Unit, San Giovanni Bosco Hospital, Torino, Italy; [5]Pneumology, [6]Department of Neurology "Amedeo Avogadvo" University, Novava

Correspondence:
Dr. Letizia Mazzini
Department of Neurology
San Giovanni Bosco Hospital Largo Donatori di Sangue 3
10154 Torino, Italy
Tel: 39 011 2402369
Fax: 39 011 2402417
E-mail: mazzini.l@libero.it

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**INTRODUCTION: Recently it has been shown in animal models of amyotrophic lateral sclerosis (ALS) that stem cells significantly slow the progression of the disease and prolong survival. We have evaluated the feasibility and safety of a method of intraspinal cord implantation of autologous mesenchymal stem cells (MSCs) in a few well-monitored patients with ALS.**

**METHOD: Bone marrow collection was performed according to the standard procedure by aspiration from the posterior iliac crest. *Ex vivo* expansion of mesenchymal stem cells was induced according to Pittenger's protocol. The cells were suspended in 2 ml of autologous cerebrospinal fluid and transplanted into the spinal cord by a micrometric pump injector.**

**RESULTS: No patient manifested major adverse events such as respiratory failure or death. Minor adverse events were intercostal pain irradiation (4 patients) which was reversible after a mean period of three days after surgery, and leg sensory dysesthesia (5 patients) which was reversible after a mean period of six weeks after surgery. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed.**

**CONCLUSIONS: Our results appear to demonstrate that the procedures of *ex vivo* expansion of autologous mesenchymal stem cells and of transplantation into the spinal cord of humans are safe and well tolerated by ALS patients.** (ALS 2003; 4: 158-161)

**Keywords:** ALS – mesenchymal stem cells – neurodegenerative – hematopoietic

## Introduction

In many adult tissues, cell loss is balanced by the proliferation and differentiation of stem cells. Recent experimental observations[1] suggest that neurodegenerative disease pathology may involve alterations in stem-cell proliferation, migration or differentiation. An increasing number of studies in animals imply that stem cells do have the ability to migrate specifically to regions of experimentally induced disease and to proliferate and differentiate into neurons and glial cells.[2,3] It has been shown that transplanted stem cells can repopulate neurons or stimulate the regeneration processes. Clinical studies with stem cells in humans with neurodegenerative disease began more than ten years ago in Huntington's disease,[5] motivated by the lack of therapy and the severity of the syndrome. Later clinical trials showed some benefit both in Parkinson's and Huntington's diseases.[6–7] ALS is characterized by selective degeneration of motor neurons which leads to progressive decline in muscular function with an unfavourable prognosis. There is no effective therapy. Novel therapeutic strategies might be directed at replacing or repairing the damaged motor neurons.

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### Hematologic background

Mesenchymal stem cells derived from adult marrow are pluripotent cells. In recent years MSCs have been used to repair damaged tissues in a number of experimental models. Our laboratory is involved in *ex vivo* expansion of hematopoietic stem cells for the treatment of young patients with hematological cancers. Our laboratory is also involved in immunological evaluation of MSCs obtained from iliac crest bone marrow, working with several growth factors in order to study the differentiation of these stem cells.[8] Recently it has been shown that they are capable of proliferating and differentiating into neurons and glial cells both *in vitro*[9–11] and *in vivo*.[12–16] Bone marrow stem cells have been transplanted in different animal models of central nervous system diseases with positive clinical effects. They ameliorate neurological deficits in rats with cerebral ischemia[17,18] and in acid sphingomyelinase-deficient mice they delay the onset of neurological abnormalities and extend their life span.[19] These data support the clinical use of these cells in order to repair central nervous system damage by locally differentiating mesenchymal stem cells into neurons.

### Stem cells in animal models of ALS

There are many difficulties in reproducing ALS in an animal model. Recently the SOD1 transgenic mouse has provided a useful experimental model of ALS although this model does not reproduce the upper and lower motor neuron syndrome characteristic of the human disease and its phases of progression. Moreover, in the SOD1 transgenic mouse damage to the motor neurons is induced by a genetic mechanism which is not the common etiological mechanism in human sporadic ALS. We believe that in stem cell research the animal models are very useful for studying the mechanism of action of the transplanted cells and the safety of the method, but are poor indicators of the clinical effects as treatment for ALS patients. As shown in many previous clinical trials with neurotrophic growth factors, the evidence of a significant positive effect in animal models was not confirmed in humans. We believe that stem cells in ALS patients might act by different mechanisms, e.g. the production of trophic factors, and the activation of motor neuron excitability by means other than replacement of neurons.

Human embryonic stem cells generate neurons when transplanted into the spinal cord of SOD1 mice[20] and the life span of SOD1 mice increased with large doses of human umbilical cord blood mononuclear cells given intravenously after 800cGy of irradiation.[21] This effect was significantly improved by doubling the dose of the cells transplanted.[22] Transplantation of stem cells derived from the human teratocarcinoma cell-line into the spinal cord of SOD1 mice slowed progression of the disease[23] and prolonged survival.[24] In a different animal model of ALS, a Sindblis virus model, the injection of human embryonic stem cells into the spinal fluid ameliorated the muscular strength of paralyzed rats.[25]

### Neurosurgical background

The choice of transplanting stem cells directly into the spinal cord was made given the impediment of stem cells to cross the blood-brain barrier. The blood-brain barrier in ALS is intact as in other degenerative and genetic diseases. Experiments performed in Parkinson's and Huntington's disease were carried out transplanting stem cells directly into the basal ganglia using a stereotaxic procedure.[6,26] A different approach has been used in other neurological diseases such as cerebral ischemia[27] or multiple sclerosis[28] in which a damage to the blood-brain barrier is recognised. Donor-derived cells with neuroectodermal characteristics were found in the central nervous system of animals after bone marrow transplantation only in conditions associated with breakdown of the blood-brain barrier.[29] These data are in accordance with our results in the SOD1 animal model in which autologous mesenchymal stem cells given both intravenously or into the spinal fluid did not reach the spinal cord and motor neurons (unpublished data). All the experiments with stem cells performed in the SOD1 ALS animal model were carried out by direct injection into the spinal cord,[20,23,24]or after the breakdown of the blood-brain barrier.[21] Furthermore no clinical effects have been observed in ALS patients who were injected intrathecally with peripheral blood stem cells.[30]

## Patients and methods

The primary objective of our study was to verify the safety and tolerability of *ex vivo* expansion of autologous mesenchymal stem cells after transplantation of stem cells directly into the spinal cord of humans. The study was approved by the Regional Ethical Committee.

The study started in October 2001. Seven patients with definite ALS (4 females and 3 males; mean age 46.6±16.8 years; range: 23–74) were recruited and treated. Patients were included if they had ALS of spinal onset with severe functional impairment of the lower limbs and mild functional impairment of the upper limbs without signs of respiratory failure (FVC > 50% and normal polisomnography). The patients were monitored by clinical evaluation which included the ALS-FRS scale, Norris score, bulbar score, and MRC strength scale. Respiratory assessment included clinical evaluation, pulmonary function tests, arterial blood gases analysis, and nocturnal cardio-respiratory monitoring. Neurophysiological assessments were made including EMG, and somatosensory evoked potentials. The neuroradiological assessment consisted of MRI of spinal cord and brain before and after Gadolinium DTA infusion. A clinical psychologist performed psychological evaluation including an interview and psychological tests.

### Mesenchymal stem cells isolation and expansion

Bone marrow (BM) was obtained by aspiration from each patient's own posterior iliac crest. BM cells were re-suspended in phosphate-buffered saline according to Pittenger's protocol.[31] The cells collected from the interface were plated in MSC medium (Bio Whittaker, Belgium) at $8 \times 10^5$ cells/cm$^2$ in T flasks (Costar, Cambridge, MA, USA) and maintained at 37°C in an atmosphere of 5% $CO_2$. After 3 days non-adherent cells were removed and replaced with fresh culture medium. Subsequent complete medium changes were performed every 4 days. After 15 days for the first passage and every 7 days for the following passages, BM cells were detached by treatment with 0.25% trypsin containing 0.01% EDTA at 10 minutes at 37°C. These cells were counted, analysed for their viability and for their immunophenotype by flow cytometry. Expansion was effected for 3–4 weeks.

The following tests on the expanded cells were carried out: 1. evaluation of sterility (absence of contamination from anaerobes and aerobic bacteria, mycetes and mycoplasma); 2. cytogenetic analysis to exclude acquired karyotype alterations; 3. evaluation of the viability and analysis of surface antigens that characterize MSCs. The adherent cells were identified by immunophenotyping analysis. Three hours before infusion, the cells were observed by optic microscope for their morphology and any eventual signs of bacterial contamination. The cells were washed and centrifuged at 200 g for 15 minutes and another wash with PBS 1X was carried out.

### Transplantation procedure

After the final wash, the cells were suspended in 2 ml of autologous cerebrospinal fluid and directly transplanted into

the surgically-exposed spinal cord at T7–T9 levels. We decided to inject stem cells at these selected levels for several reasons: 1. from a surgical viewpoint the risk of inducing an iatrogenic neuronal spinal cord injury is lower than at the more rostral levels; 2. the hypothesized neuronal reinnervation at these levels might have a greater chance of being detected because of the shorter distance between spinal cord and muscle; 3. any evidence for an increase in muscular recruitment cannot be the result of the iatrogenic spinal cord injury, e.g. muscular hypertonia. Thus a change in functional performance at higher muscle levels cannot be related to the surgical procedure.

CSF was preferred as buffer as it offers the best milieu for the stem cells. The procedure was performed under general anesthesia using short-acting drugs. The patient was placed in the prone position on the operating table and the level of incision was verified with fluoroscopy. A median linear skin incision was made at the T6–T9 level and the corresponding vertebrae were identified. Following this a laminectomy was performed at T9–T8, and if necessary T7, and the surgical microscope was introduced. The dura was opened along the median line and the cells were injected in the most central part of the spinal cord by means of the Hamilton syringe previously mounted in an injection system with a micrometric pump injector supported by a table-fixed arm. At the end of the procedure the dura was closed in a watertight manner in order to avoid CSF leakage. The incision was closed in a standard fashion.

## Results

No patients manifested severe adverse events such as death, respiratory failure or permanent post-surgical neurological deficits. Minor adverse events were: intercostal pain (4 patients) which was reversible after a mean period of 3 days (range: 1–6) after surgery, leg sensory dysesthesia (5 patients) which was reversible after a mean period of 6 weeks (range: 1–8) after surgery. No patient manifested bladder and bowel dysfunction, or leg motor deficit. There were no anesthetic complications. MRI with Gadolinium DTA infusion performed at 3 and 6 months after implantation showed no evidence of structural changes of the spinal cord or signs of abnormal enhancement indicative of a possible abnormal cell proliferation when compared with the baseline. Somatosensory evoked potentials from tibial nerve stimulation showed a mild delay of the central conduction time 3 days after surgery but this normalized 1 month after transplantation.

All patients showed a good acceptance of the procedure and no significant modifications of the psychological status or quality of life were observed. Muscular strength (MRC scale) declined in the six months before transplantation in all patients. At the third month after stem cell implantation a trend towards a slowing down of the linear decline of muscular strength was evident in 4 patients in the proximal muscle groups of the lower limbs, while in 2 patients a mild increase in strength was observed in the same muscle groups.

## Conclusions

Our results appear to demonstrate that the procedures of *ex vivo* expansion of autologous mesenchymal stem cells and of transplantation of these cell suspensions into the spinal cord of humans are safe and well tolerated by ALS patients. This study was essentially concerned with technological aspects of this procedure. No placebo procedures were carried out. Given the progressive decline of muscular strength characteristics of ALS the results obtained are encouraging.[32] Our observations allow no comment to be made on the possible efficacy of stem cells in the therapy of ALS patients; controlled studies will be necessary to evaluate such usage. The minimal side effects and the absence of detrimental effects on neurological function support further research in stem cell transplantation in carefully monitored patients with ALS. Moreover, the same procedures could be used to deliver nerve growth factors in stem cells into the spinal cord of patients with ALS, thereby avoiding the complications of systemic delivery.

## References


1. Armstrong RJE, Barker R. Neurodegeneration: a failure of neuroregeneration? Lancet 2001; 358: 9288.
2. McDonald JW, Liu XZ, Qu Y et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 1999; 5: 1410–1412.
3. Liu S, Stewart TJ, Howard MJ et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 2000; 97: 6126–6131.
4. Bjorklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. Nature Neuroscience 2000; 3: 537–544.
5. Lindvall O. Prospects of transplantation in human degenerative disease. Trends in Neuroscience 1991; 14(8): 376–384.
6. Freed CR, Greene PE, Breeze RE et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001; 344: 710–719.
7. Dunnett SB, Bjorklund A, Lindvall O. Cell therapy in Parkinson's disease-stop or go? Nat Rev Neurosci 2001; 2: 365–369.
8. Mareschi K, Biasin E, Piacibello W et al. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica 86 2001: 1099–1100.
9. 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: 247–256.
10. Kim BJ, Seo JH, Bubien JK, Oh YS. Differentiation of adult bone marrow stem cells into neuroprogenitor cells in vitro. Neuroreport 2002; 13(9): 1185–1188.
11. Woodbury D, Reynolds K, Black IB. Adult bone marrow stromal stem cells express germline, ectodermal, endodermal, and mesodermal genes prior to neurogenesis. J Neurosci Res 2002; 15; 69(6): 908–917.

12. Fukunaga A, Uchida K, Hara K et al. Differentiation and angiogenesis of central nervous system stem cells implanted with mesenchyme into ischemic rat brain. Cell Transplant 1999; 8(4): 435–441.
13. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neuroscience Res 2000; 61: 364–370.
14. Black IB, Woodbury D. Adult rat and human marrow stem cells differentiate into neurons. Blood cells, Molecules and diseases 2001; 27: 3.
15. Terada N, Hamazaki T, Oka M et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002 6880; 416: 542–545.
16. Jang Y, Jahagirdaz BN, Reinbardt RL et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002 6893; 418: 41–43.
17. Zhao LR, Duan WM, Reyes M et al. Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Experimental Neurology 2002; 174: 1.
18. Chen J, Li Y, Wang L et al. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J of the Neurological Sciences 2001; 189: 1–2; 49–57.
19. Jin HK, Carter JE, Huntley GW, Schumanchman EH. Intracerebral transplantation of mesenchymal stem cells into acid sphingomyelinase-deficient mice delays the onset of neurological abnormalities and extends their life span. The J of Clinical Investigation 2002; 109, 9: 1183–1191.
20. Maragakis NJ, Teng YD, Kerr D et al. Transplanted neural stem cells are capable of engraftment and differentiation in transgenic mutant SOD1 mice. Soc Neurosci Abs 2000; 668: 3.
21. Ende N, Weistein F, Chen R et al. Human umbilical cord blood effect on SOD mice amyotrophic lateral sclerosis. Life Sci 2000; 67: 53–59.
22. Chen R, Ende N. The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. J Med 2000; 31: 21–30.
23. Garbuzova-Davis S, Willing AE, Milliken M et al. Intraspinal implantation of hNT neurons into SOD1 mice with apparent motor deficit. In: Amyotrophic Lateral Sclerosis and Other motor neuron disorders 2001; (4): 175–180.
24. Garbuzova-Davis S, Willing AE, Milliken M et al. Positive effect of transplantation of hNT neurons (NTera 2|D1 Cell-Line) in a model of familial amyotrophic lateral sclerosis. In: Experimental Neurology 2002; 174(2): 169–180.
25. Vastag B. Stem cells step closer to the clinic: paralysis partially reversed in rats with ALS-like disease. JAMA 2001; 285(13): 1691–1693.
26. Lindvall O, Hagell P. Cell therapy and transplantation in Parkinson's disease. Clin Chem Lab Med 2001; 39(4): 356–61.
27. Kondziolka D, Wechsler L, Goldstein S et al. Transplantation of cultured human neuronal cells for patients with stroke. Neurology 2000; 55: 565–569.
28. Mancardi GL, Saccardi R, Filippi M et al. Autologous hematopoietic stem cell transplantation suppresses Gd-enhanced MRI activity in MS. Neurology 2001; 10; 57(1): 62–68.
29. Jiang Y, Jabagivdar BN, Reinbardt RL et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; Jul4; 418 (6893): 41–49.
30. Janson CG, Ramesh TM, During MJ et al. Human intrathecal transplantation of peripheral blood stem cells in amyotrophic lateral sclerosis. J Hematother Stem Cell Res 2001; 10: 913–915.
31. Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–147.
32. Munsat TL. Issues in clinical trial design I: use of natural history controls in therapeutic trials. A protagonist view. Neurology 1996; 47 (Suppl 2): S96–97.