Stem cells in multiple sclerosis

Bassem I. Yamout, MD, FAAN

Professor of Clinical Neurology

Head, Multiple Sclerosis Center Clinical Research

American University of Beirut Medical Center

September 15, 2012

The Salim El-Hoss Bioethics and Professionalism Program

3rd regional conference

Stem Cell Research: Current Controversies

Definition

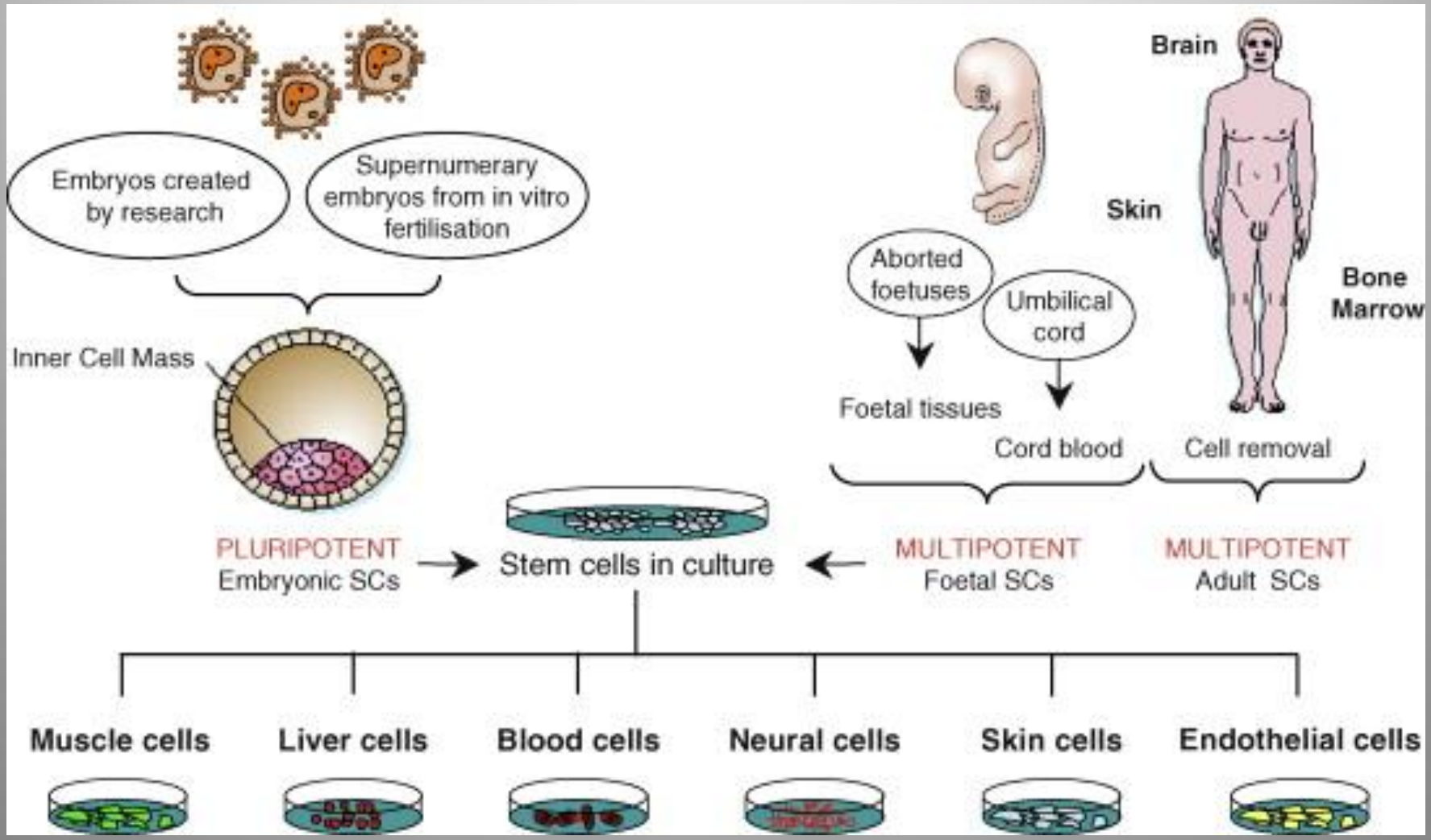
- **The classical definition of a stem cell requires that it possesses two properties:**

  ***Self-renewal* - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.**

  ***Potency* - the capacity to differentiate into specialized cell types**

- **Pluripotent stem cells can differentiate into nearly all cells,i.e. cells derived from the inner cell mass of the embryo.**
- **Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.**

Sources of Stem Cells



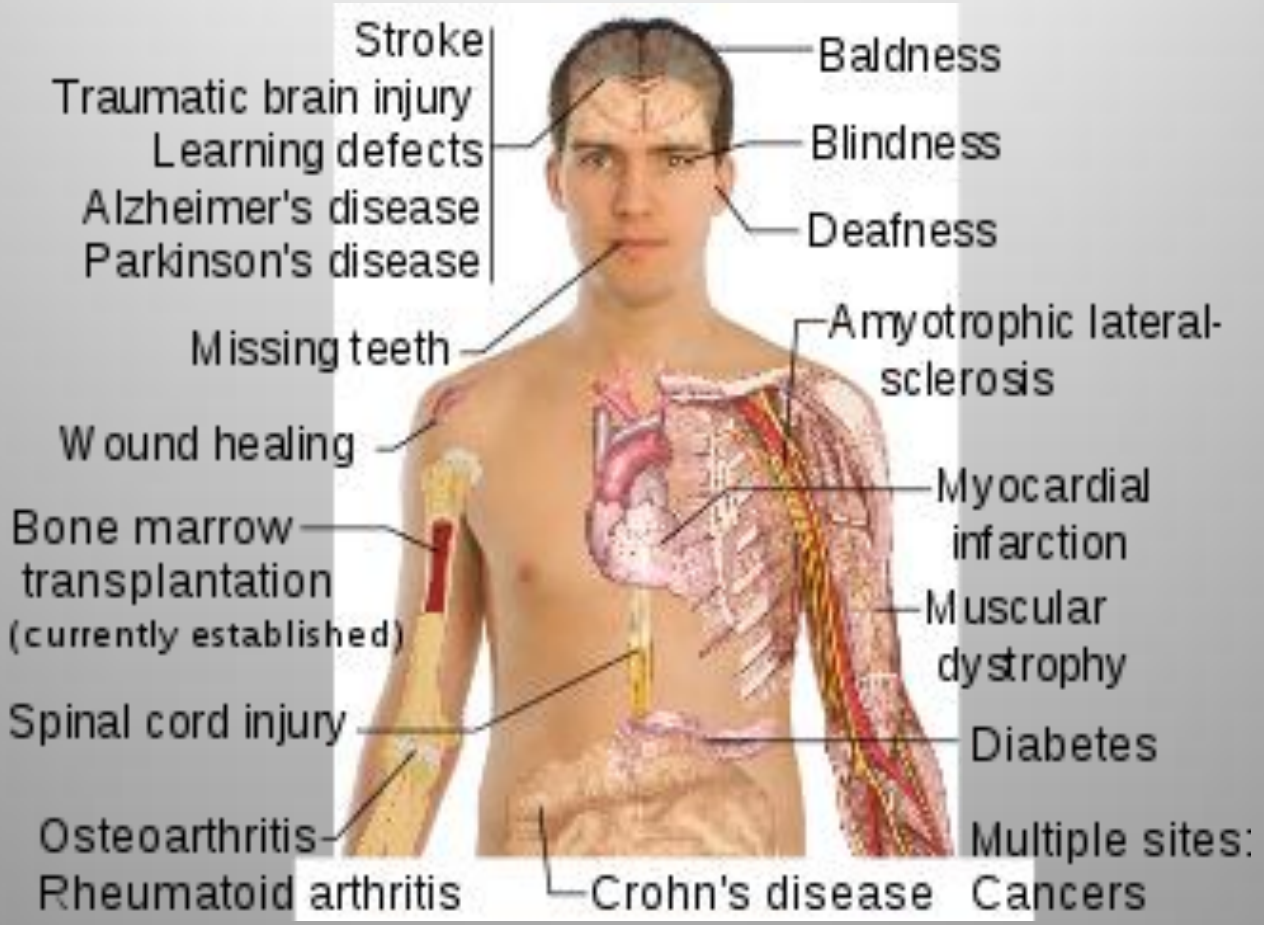
Embryonic Stem Cells

- **Disadvantages :**
  - **Risk of teratoma formation**
  - **Ethical issues**
- **Advantages :**
  - **Ability to differentiate into myelin-producing cells and neurons**

Adult Stem Cells

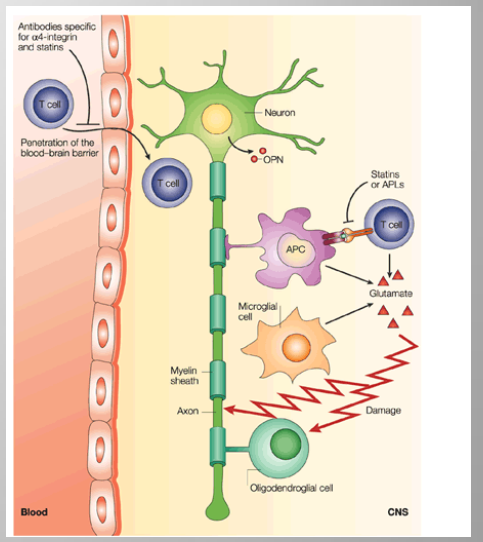
- **Advantages :**
  - **Present in different tissues like bone marrow, adipose tissue, olfactory bulb, central nervous system and others.**
  - **Easy to obtain from autologuous donors**
  - **Do not pose any ethical problems**
  - **Have not been shown to induce tumor formation in different animal models**
- **Disadvantages :**
  - **No convincing evidence of differentiation into functional mature nerve cells**

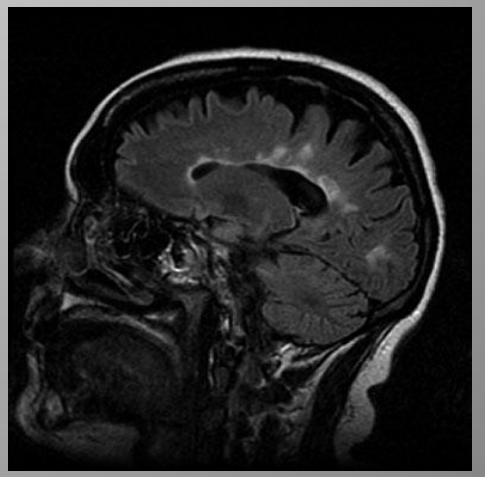
Potential uses of stem cells



Multiple Sclerosis

- Multiple sclerosis was first described by Charcot in 1868
- MS is the most common non-traumatic cause of neurologic disability in young adults. It is generally believed to be an autoimmune condition in which autoreactive T cells attack myelin sheaths leading to demyelination and axonal damage.
- Failure of the repair mechanisms is a major contributing factor to the ultimate outcome in MS.
- The most common form starts with remitting-relapsing symptomatology and minimal disability initially, leading in 10-15 years to a progressive form with a downhill course and disability accumulation.
- Currently available medical therapies are only partially effective.
- An ideal treatment would reduce the abnormal immune response and enhance repair through boosting intrinsic repair mechanisms or even cell replacement.





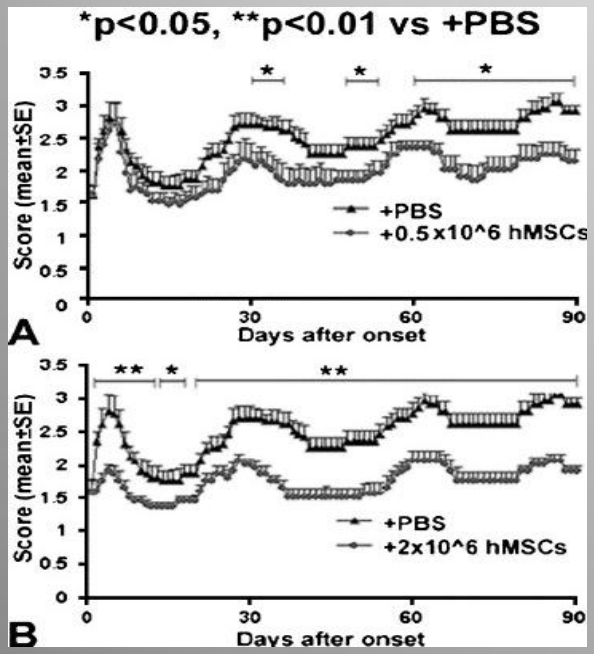
Mesenchymal Stem Cells in Multiple Sclerosis

Animal Experiments

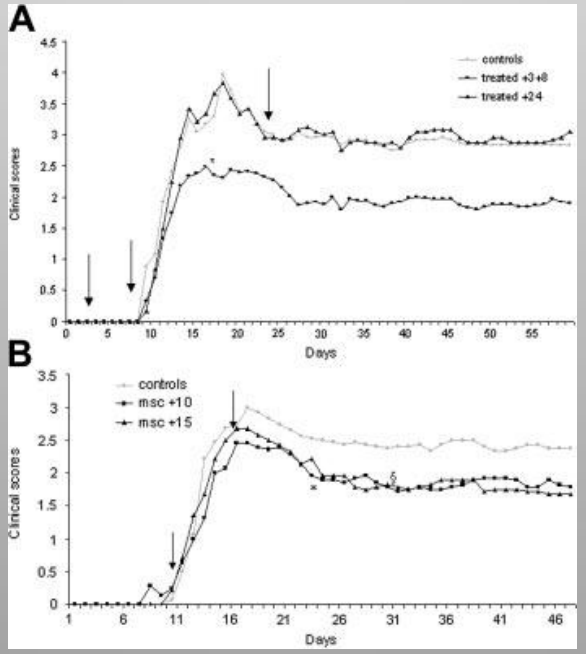
Animal Experiments

- **BMSC injected into rats with EAE, the animal model of MS, have better clinical outcomes**

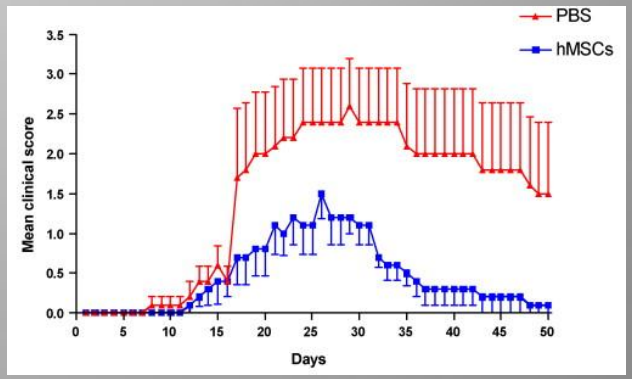
- **Clinical improvement is achieved with different routes of injection: intravenous, intraventricular and intraperitoneal**



Zhang et al.



Zappia et al.

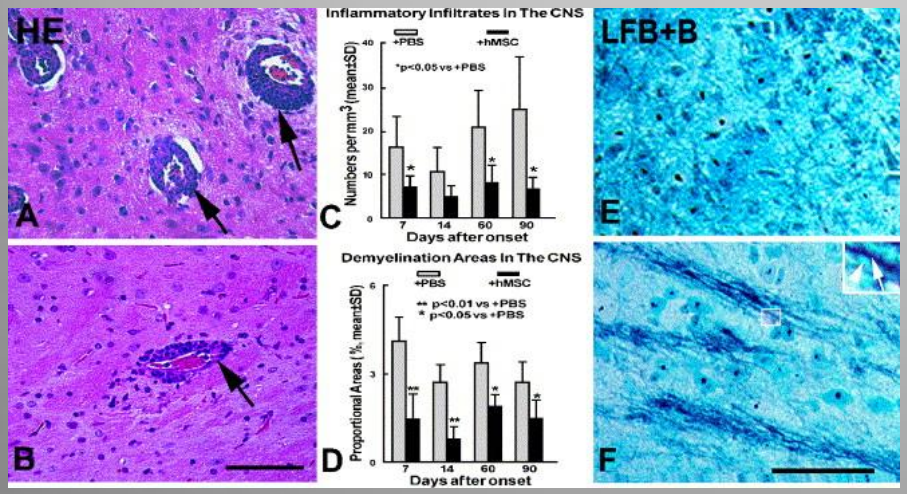


Gordon et al.

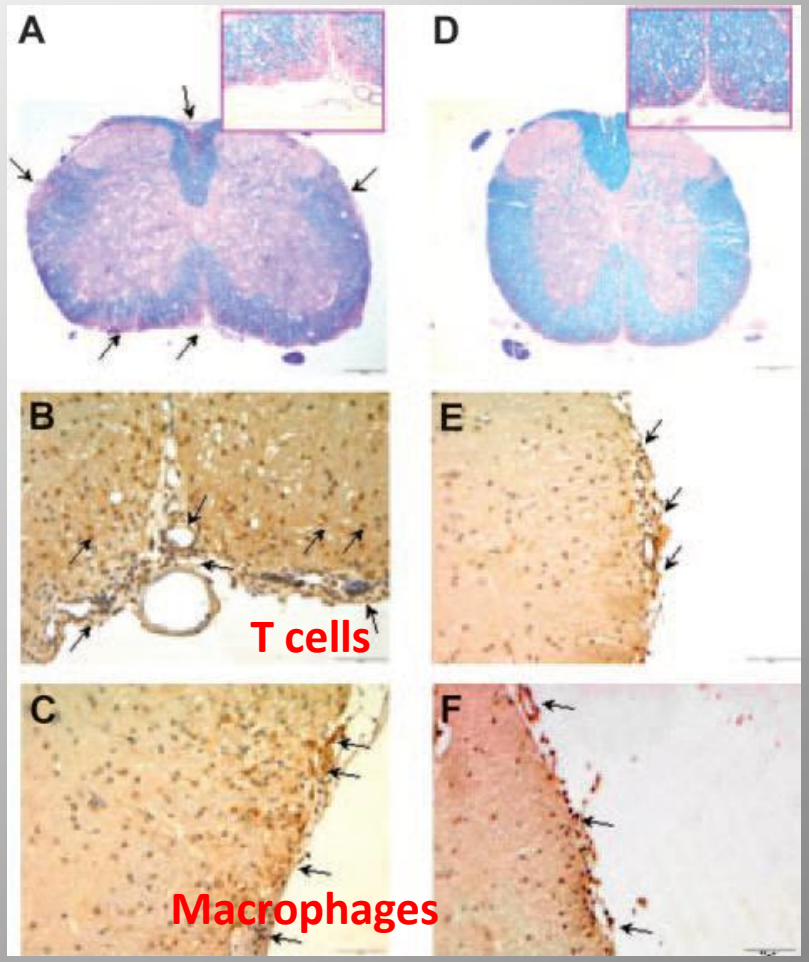
Mechanisms of Action of Mesenchymal Stem Cells

Animal Experiments

- CNS pathology shows decreased demyelination and inflammatory infiltrates pointing towards an immunomodulating effect
- BMSC administered systemically localize in peripheral lymphoid organs as well and exert a peripheral immunomodulating effect



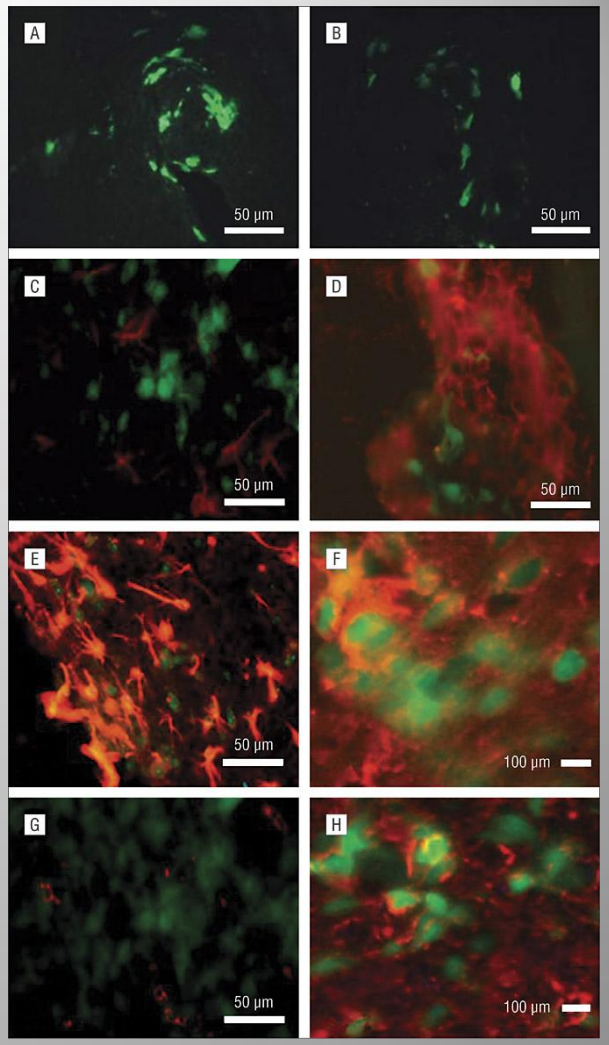
Zhang et al.



Zappia et al.

Animal Experiments

BMSC can play a role in neuroregeneration.They migrate into the CNS and differentiate into neuron-like cells expressing neuronal, astrocytic and oligodendrocytic markers. Their actual contribution to cell replacement is still controversial , since it was never convincingly shown that those cells were functional.

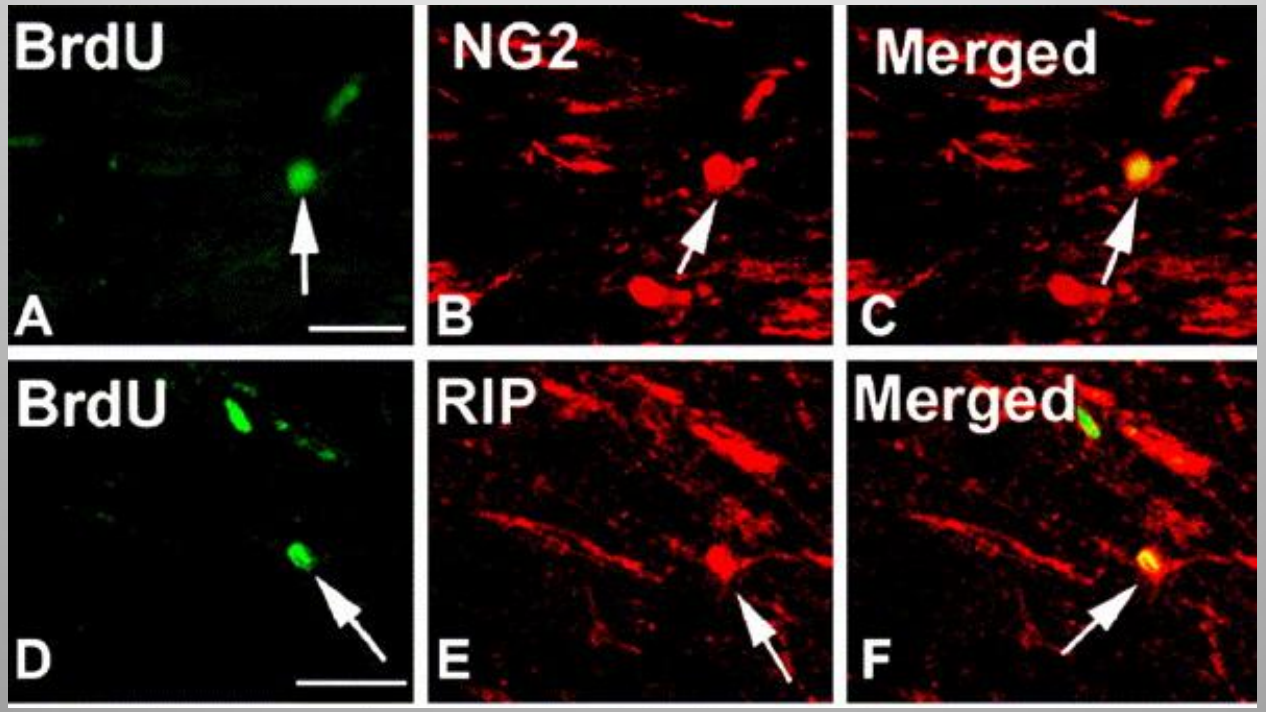


Mesenchymal stromal cells (MSCs) from syngeneic green fluorescent protein (GFP)–transgenic donors were injected intravenously (A, C, E, and G) or intraventricularly (B, D, F, and H) into mice on day 10 following induction of chronic experimental allergic encephalitis. The GFP-positive cells (A and B) were costained with the neural marker **β-tubulin type III (C and D)** , the astrocytic marker glial fibrillary acidic protein: **GFAP (E and F)** , and the oligodendrocytic markers O4 (G) and **galactocerebroside (H)**. The GFP-positive cells appear green and the differentiation marker–positive cells appear red (rhodamine-conjugated antibodies).

Kassis et al.

Animal Experiments

hBMSC can enter the CNS and induce proliferation of local progenitor cells , enhancing the remyelinating process

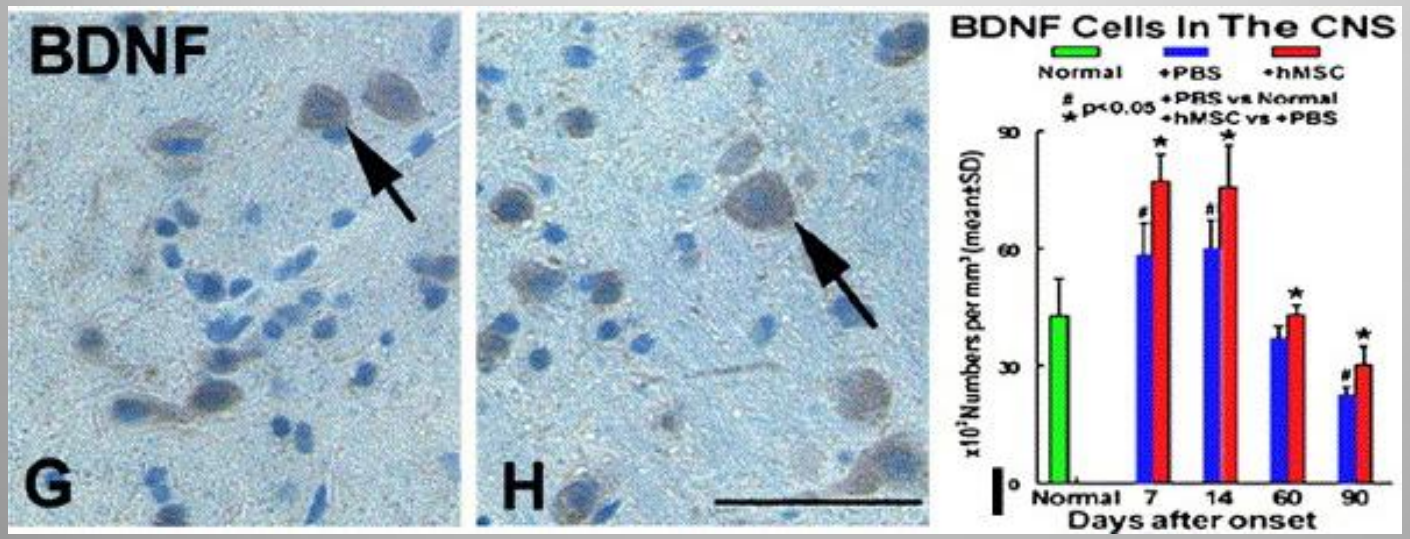


Double immunofluorescence staining revealed that $BrdU^{+}$ (proliferation marker)cells (FITC, green, A, D) were reactive for NG2 (OPC marker) (CY3, red, B–C) and RIP (Oligodendrocyte marker) (CY3, red, E–F).

Zhang et al.

Animal Experiments

hBMSC can promote neuroprotection by secreting and promoting production of bioactive trophic factors in the CNS, leading to inhibition of gliosis, scar formation and apoptosis.



Immunohistochemical staining (DAB, brown; hematoxylin, blue) showed BDNF$^+$ cells in the striatum of EAE mice treated with PBS (G) or hBMSCs (H). Quantitative data showed number of BDNF$^+$ cells in the CNS significantly increase at 7, 14, 60, and 90 days in hBMSC-treated mice compared with EAE control mice (I).

Zhang et al.

Conclusions from animal experiments

- **BMSC are effective in EAE. They improve clinical outcome, preserve axons, decrease inflammation and reduce demyelination.**
- **BMSC exert their beneficial effects through 3 mechanisms: immunomodulation, neuroprotection and neuroregeneration.**
- **The immunomodulatory effect is through the peripheral lymphoid organs and probably locally in the brain both by cell-to-cell contact and release of anti-inflammatory molecules (IL-1R antagonists...). They inhibit T and B cells proliferation as well as antigen presenting cells maturation.**
- **BMSC can promote neuroprotection by secreting and promoting production of bioactive trophic factors in the CNS, leading to inhibition of gliosis, scar formation and apoptosis.**
- **BMSC can play a role in neuroregeneration.They migrate into the CNS and differentiate into neuron-like cells expressing neuronal , astrocytic and oligodendrocytic markers. Their actual contribution to cell replacement is still controversial , since it was never shown convincingly that those cells were functional. They can however induce proliferation of local progenitor cells.**

Mesenchymal Stem Cells in Multiple Sclerosis

Human Trials

Safety and Feasibility of Autologous Bone Marrow Cellular Therapy in Relapsing-Progressive Multiple Sclerosis

CM Rice[1], EA Mallam[1], AL Whone[1,2,3], P Walsh[1], DJ Brooks[2], N Kane[1], SR Butler[3], DI Marks[4] and NJ Scolding[1]

- Six relapsing-progressive MS patients with ≥ 1 relapse within the past 2 years.
- Bone marrow harvested from iliac crest under general anesthesia was infused intravenously without processing or cell expansion.
- Patients were followed for 1 year with clinical assessments, multimodal evoked potentials, and brain MRI scans.
- Three patients experienced moderate adverse events following the procedure including temporary urinary retention in one and transient increase in lower limb spasticity in two.
- EDSS showed no change 1 year post-therapy (+0.1 mean change).
- All components of the MSFC showed slight improvement after 1 year which was not statistically significant.
- Global EP score showed statistically significant improvement at 1 year
- MRI lesions increased at 3 weeks then stabilized by 3 months

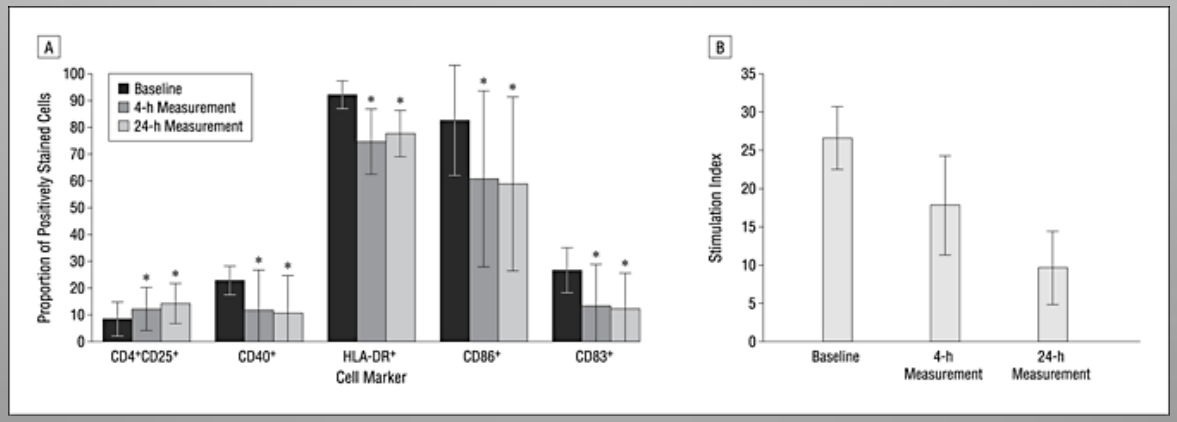
	Mean change at 1 year
EDSS	+0.1
25-ft walk	-0.19 s
9-Hole peg test	-1.43 s
PASAT	+7.17
Global EP score	+2.5

Clinic Pharmacol Ther. 2010 Jun;87(6):679-85

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

- 15 MS patients were injected intrathecally with in-vitro expanded autologous mesenchymal stem cells(mean of 63.2×10^6 cells), 5 of whom received an additional intravenous injection(mean of 24.5×10^6).
- Short febrile reactions were reported in 10 patients, and LP-related headaches in another 10. One patient had aseptic meningitis probably secondary to residual chemicals in the injected medium.
- Mean EDSS improved from 6.7 to 5.9 at 6 months (unchanged in 4 and improved in 11 patients by 0.5-2.5 points). *P=0.001*
- Brain MRI at 48 hours, 1,3, and 6 months revealed no new or Gd+ lesions



Arch Neurol. 2010 Oct;67(10):1187-94

Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study

Peter Connick, Madhan Kolappan,* Charles Crawley, Daniel J Webber, Rickie Patani, Andrew W Michell, Ming-Qing Du, Shi-Lu Luan, Daniel R Altmann, Alan J Thompson, Alastair Compston, Michael A Scott, David H Miller, Siddharthan Chandran*

- 10 SPMS patients were injected intravenously with in-vitro expanded autologous mesenchymal stem cells(mean of $1.6x10^6$ cells/Kg) and followed for 10 months.
- 1 patient had a rash and 2 patients developed self-limiting bacterial infections.
- There was improvement in visual acuity *(P=0.003),* visual evoked responses *(P=0.02),* and optic nerve area *(P=0.006).*
- There was no significant effects on color vision, visual fields, macular volume, retinal nerve fibre layer thickness, or optic nerve magnetization transfer ratio.
- EDSS improved *(P=0.028)* but MSCFC was unchanged.
- MRI T2 and T1 lesion loads were unchanged
- The evidence of structural, functional, and physiological improvement after treatment in some visual endpoints is suggestive of neuroprotection.

Lancet Neurol. 2012 Feb;11:150-6

Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study

B Yamout, R. Hourani, H. Salti, W. Barada, T. El -Hajj, G. Skaff, A. Koutobi, A Herlopian, E. Bazz, R. Mahfouz, R. Khalil-Hamdan, N. Kreidyeh, M. El-Sabban, A. Bazarbachi

American University of Beirut Medical Center (Beirut, LB)

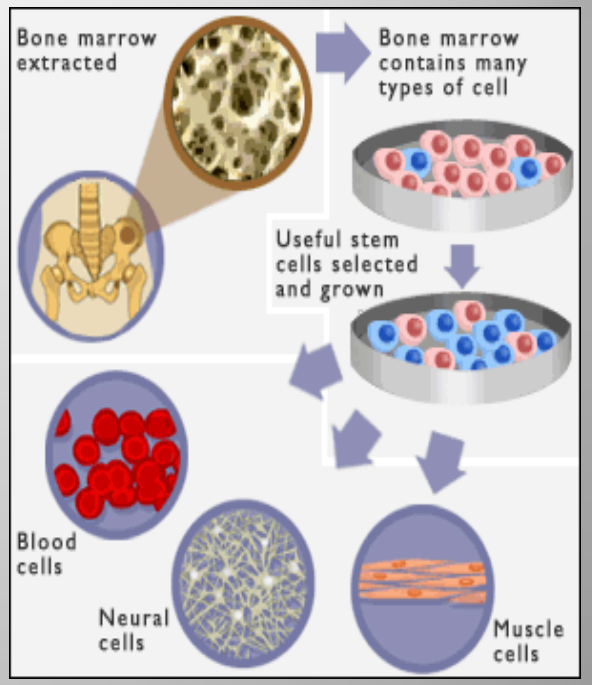
Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

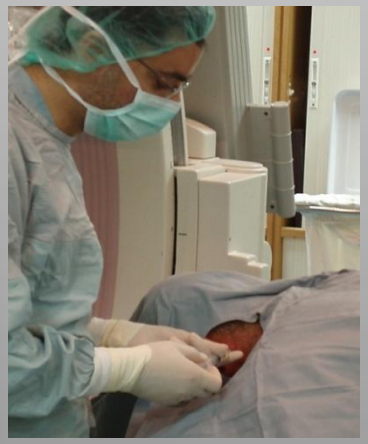
Experimental Design

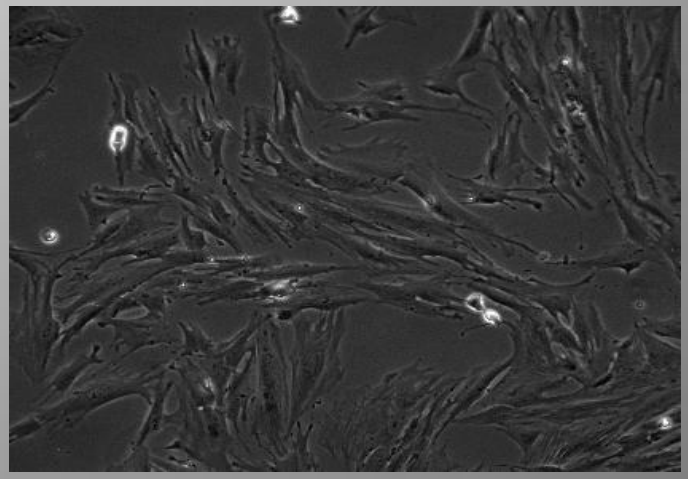
- Subjects with clinically definite MS, aged between 18 and 65 years, failing medical therapy
- EDSS : 4.0 -7.5
- No evidence of bone marrow disease
- Clinical assessement: EDSS and MSFC at baseline , 3 and 12 m.
- MRI assessement: Baseline, 3 and 12 m:
  - Brain imaging: Sagittal and axial T2 and proton density, sagittal T2, coronal FLAIR, axial T1, axial FLAIR and axial T1 images after gadolinium administration
  - Single voxel brain MR spectroscopy.
  - Cervical and thoracic spine axial and sagittal T2 and T1 images after gadolinium administration .
  - Optic nerves coronal T2 images.
- Visual function and retinal nerve fiber layer (RNFL) assessment: baseline, 3 and 12 m :
  - Best corrected visual acuity using the distance ETDRS charts.
  - Contrast sensitivity using the Sloan contrast sensitivity charts at 2.5% and 10%.
  - Nerve fiber layer thickness was calculated using the Zeiss Stratus OCT 3, version 4.0.1 software.

Stem cell processing

- BM is harvested from iliac bone.
- Mononuclear cells are isolated by Ficoll density centrifugation.
- Mononuclear cells (1 x 10^6/ml) are cultivated in low-glucose medium containing 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator at 37° C under 5% CO_2. Attached cells that develop into colonies within 5 to 7 days are harvested using 0.25% trypsin and sub-cultured.
- These autologous mesenchymal stem cells are expanded (for 6-8 weeks) to reach 20-100 x 10^6 cells. Cell viability is determined by trypan blue staining and cell cultures are tested weekly for sterility.
- The surface expression of MSC markers [CD29+, CD44+, CD166+, CD45+, CD34-, CD14-] on culture-expanded MSC is measured
- Demonstration of in-vitro differentiation into osteoblasts and chondrocytes.
- 20-100 x 10^6 cells are recovered and injected into the lumbar and cisternal CSF.







Patient demographics

Patient	Course	Age/Sex (y)	Disease Duration (y)	Medical Therapy	Number of BMSc (10^6)
1	SPMS	38/M	11	BINF	100
2	SPMS	38/F	15	BINF/MTX/AZA	42
3	SPMS	44/M	18	BINF/AZA/MTX	52
4	SPMS	49/M	31	BINF/CPX/MET	32
5	SPMS	44/F	22	BINF/MTX	33
6	SPMS	47/F	27	BINF	36
7	RRMS	43/F	27	BINF	1.1
8	SPMS	35/F	25	MTX/CPX	1.5
9	SPMS	34/F	15	BINF	34
10	SPMS	56/M	24	BINF/MTX/	1.5

Adverse Events

- The only major adverse event was a transient encephalopathy with seizures in patient 1, occurring few days after cell injection. He required hospitalization and intravenous valproate, but recovered without significant sequelae. He was injected with the highest number of MSC (100x 10^6). The remaining 6 patients received 32-52x 10^6 cells

- Patient 3 had transient cervical and low back pain for few days without fever or meningeal signs.

Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

Results-EDSS

Patient	Baseline	3-6 Months	12 Months
1	7.5	8.0	7.0
2	6.5	6.0	6.0
3	4.5	4.0	4.5
4	7.0	7.0	7.0
5	6.5	5.5	6.5*
6	7.0	6.5	6.5
9	6.5	6.0	NA
Mean	**6.5**	**6.14**	**6.25**

- The EDSS improved by 0.5 in 4 patients, and was unchanged in 3 patients.

** Patient 5 developed multiple myeloma with multiple vertebral fractures that limited ambulation*

Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

Results- Clinical Parameters

	Baseline	3-6 m	12 m	1 year change
EDSS *Mean (SD)*	6.5 (0.9)	6.14 (1.24)	6.25 (0.93)	- 0.25
8 m walk *Mean (SD)*	40.1 (41.1)	36.4 (20.5)	23.5 (26.2)	- 16.6
9-Hole peg test *Mean (SD)*	36.6 (16.8)	30.6 (11.6)	23.3 (4.4)	-13.3
PASAT *Mean (SD)*	22.1 (12.2)	27.9 (14.7)	35.7 (14.0)	+13.6
Sloan Contrast Sensitivity2.5% *Mean (SD)*	_	+0.92 (2.75)	+2.25 (1.83)	+2.25
OCT (RNFL thickness *um*) *Mean (SD)*	34.5 (1.3)	31.75 (4.03)	35 (2.0)	+0.5

Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

Results-MRI

	Baseline	3-6 m	12 m*	1 year change
Gd+ lesions/pt *Mean (SD)*	0 (0)	1.14 (1.86)	0.33 (0.58)	+0.33
NAA/Cr *Mean (SD)*	2.13 (0.47)	1.96 (0.24)	1.85 (0.50)	- 0.28

* 12 m MRI was available on 4 patients

Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

Conclusions

- **We showed that treatment of MS patients with autologuous in-vitro expanded BMSC is feasible and safe up to 1 year of follow-up.**
- **Thirty percent of patients failed to grow adequate number of BMSC ($<2x10^6$) inspite of repeated bone marrow aspirations, possibly due to previous immunotherapies.**
- **There were some indications of efficacy although the numbers were too small to draw any definite conclusions.**
- **All patients had evidence of progression in the year preceding enrollement in the trial, yet at 3-6 months post-treatment most patients showed improvement on different components of the EDSS, MSFC and visual testing. Clinical improvement was maintained in most patients at 1 year.**
- **MRI showed an increase in enhancing lesions in some patients, which might reflect an inflammatory reaction to the transplanted cells rather than disease activity.**
- **The decrease in NAA/Cr ratio in most patients reflects ongoing axonal degeneration.**

Yamout et al. J Neuroimmunol; Oct 2010, 227: 185

Many questions still need to be answered !

- **What is the exact mechanism of action of BMSC, and is it predominantly peripheral or central?**
- **What is the ideal route of administration?**
- **What is the number of cells needed?**
- **Should we use it in patients with advanced disability only, or rather in relapsing remitting cases at an earlier stage, when therapeutic interventions are usually more effective?**
- **How frequently should this therapy be given to maintain its effect in a chronic disease such as MS?**
- **Will there be unforseen longterm adverse events?**

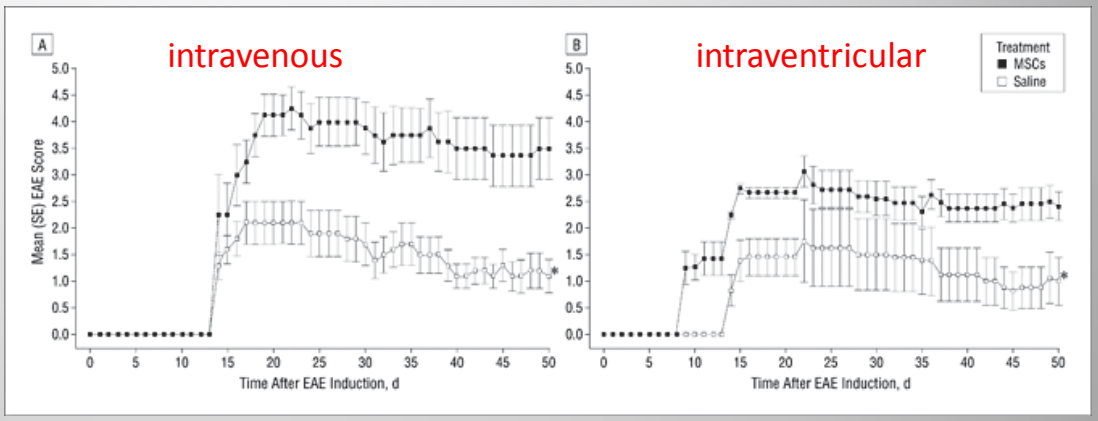
An international multicenter placebo-controlled trial is needed to answer all those questions

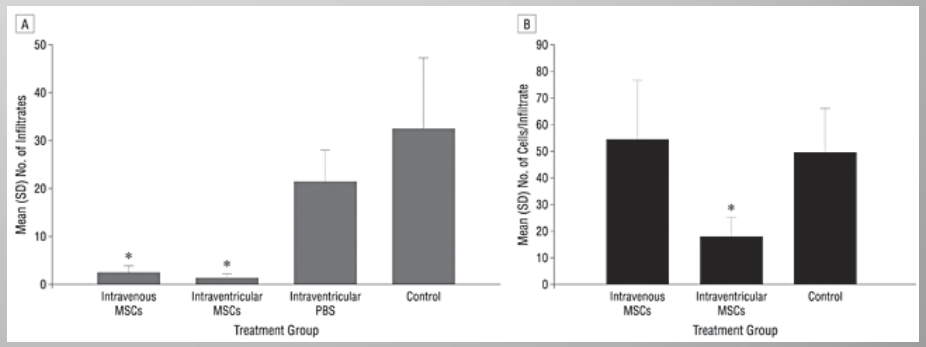
THANK YOU

* This study was funded by intramural grants as well as grants from the “Azem and Saadeh” non-profit Lebanese association

Animal Experiments

- **The only study comparing intravenous and intraventricular routes of administration of BMSC in EAE showed that direct injection into the ventricles induced a more pronounced reduction in Infiltrating cells and increased preservation of axons**





Treatment Group	Axonal Injury, Mean (SE), %				
	Normal	Few Scattered	Focused Mild to Moderate	Scattered Mild to Moderate or Focused Severe	Scattered Severe
Control	40 (5)	26.7 (5)	10.0 (5)	20.0 (5)	3.3 (5)
Mice with EAE					
Intraventricular PBS	56.4 (5)	23.1 (5)	5.1 (5)	12.8 (5)	2.6 (5)
Intravenous PBS	46.0 (5)	30.0 (5)	10.0 (5)	10.0 (5)	4.0 (5)
Intraventricular MSCs	95.8 (5)[b]	4.2 (5)[b]	0	0	0
Intravenous MSCs	85.0 (5)[c]	10.0 (5)[c]	5.0 (5)	0	0

Kassis et al.

Results-clinical

- **At 6 months, the 8 meter walking time in the 3 ambulatory patients showed improvement in 2 patients (from 100 to 56 and 8 to 6 sec respectively), maintained at 1 year, and worsening in 1 (from 31 to 55 sec).**
- **The PASAT score improved at 3-6 months in 6 patients and worsened in one (from a mean of 22 to 28). In the 3 patients with 1 year follow-up, this initial improvement was still maintained.**
- **The 9-peghole test results at 3-6 months showed improvement in 5, stabilization in 6, and worsening in 2 extremities. The 1 year data available on 3 patients was similar .**

Results-visual testing

- **Visual testing available at 3 months on 6 patients showed improvement by 1-3 lines in 5/6 and worsening by 1 line in1/6 patients.**
- **RNFL thickness by OCT, available on 4 patients, was unchanged in 3 and decreased by 24% in 1 Patients.**
- **In 3 patients with 12 months follow-up, visual testing showed further improvement in 2 and slight worsening in 1 who was still improved compared to baseline., but the RNFL thickness was unchanged.**

Patient		Baseline	3 Months	12 Months
1	OCT*	35	32	35
	Sloan Contrast Sensitivity 2.5% (OD/OS)+	-	+3/+3	+1/+1
2	OCT	36	35	37
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-	+2/+2	+4/+2
3	OCT	33	34	33
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-/FC	+2/20:60$	+5/20:60$
4	OCT	34	26	
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-	-4/-5	-
5	OCT	-	-	-
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-	+1/0	+1/0
6	OCT	-	-	-
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-	-	-
9	OCT	-	-	-
	Sloan Contrast Sensitivity 2.5% (OD/OS)	-	+1/+2	-

Results-MRI

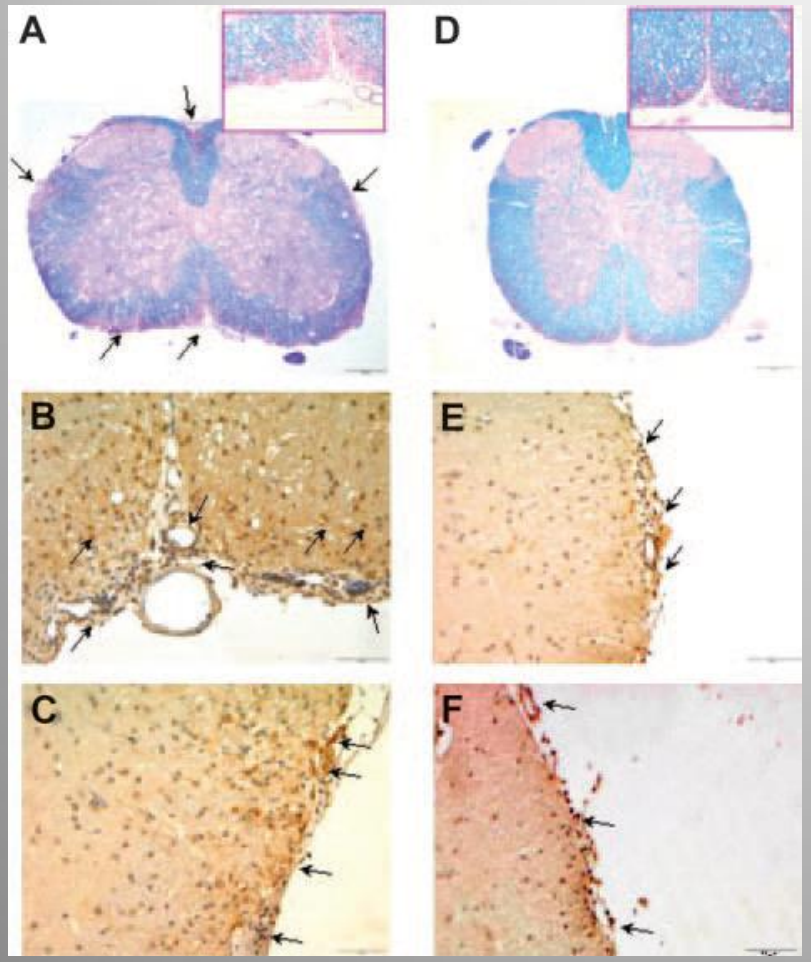
- **MRI data at 3 months revealed Gd+ lesions in 3/7 patients as opposed to 0/7 at baseline**
- **MRS was available on 6 patients and revealed a decrease in the NAA/Cr ratio by a mean of 0.18. In the 3 patients with data at 1 year, 2 showed stabilization and the other further decrease in the NAA/Cr ratio.**

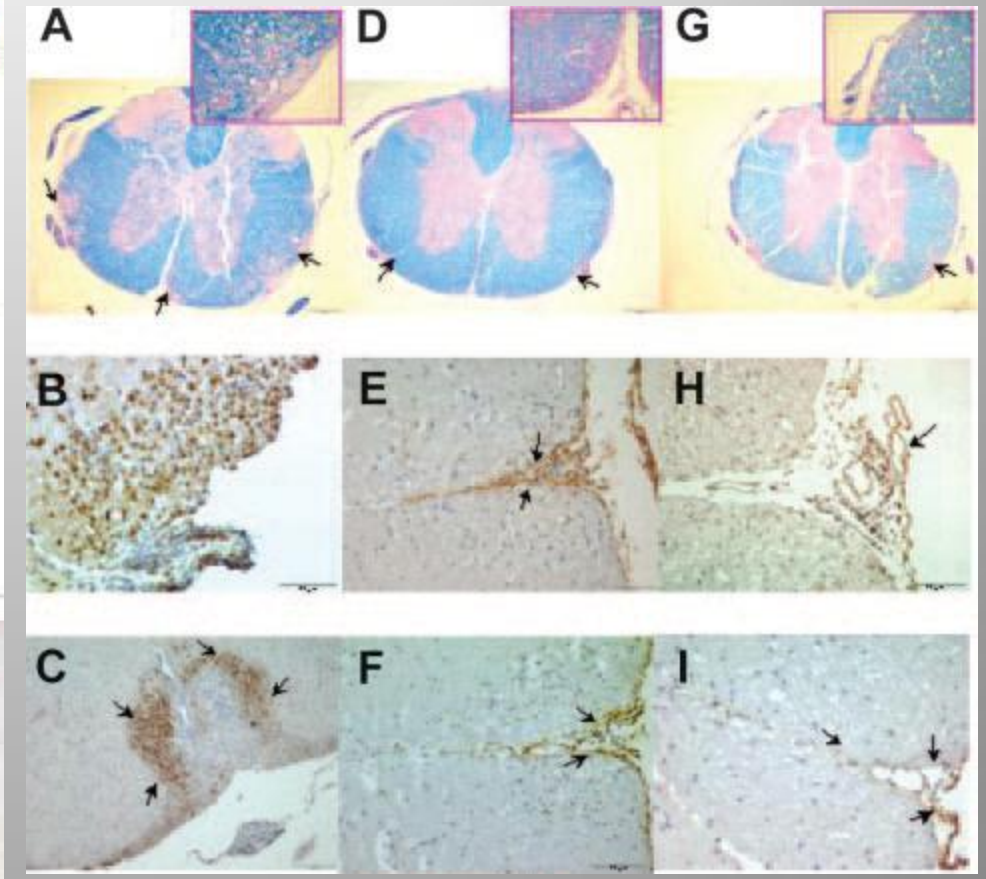
Patient		New/Enlarging Lesions*	Gd^+ Lesions	NAA/CR
1	Baseline	-	0	2.5
	3Months	3	0	2.0
	12 Months	1	0	NA
2	Baseline	-	0	2.6
	3Months	5	0	2.3
	12 Months	2	1	1.5
3	Baseline	-	0	2.5
	3Months	0	2	2.1
	12 Months	0	0	2.2
4	Baseline	-	0	2.0
	3Months	9	5	2.0
	12 Months	NA	NA	NA
5	Baseline	-	0	NA
	3Months	0	0	2.0
	12 Months	0	0	2.0
6	Baseline	-	0	1.5
	3Months	1	1	1.7
	12 Months	NA	NA	NA
9	Baseline	-	0	1.7
	3Months	1	0	1.6
	12 Months	NA	NA	NA

Current Human Experiments

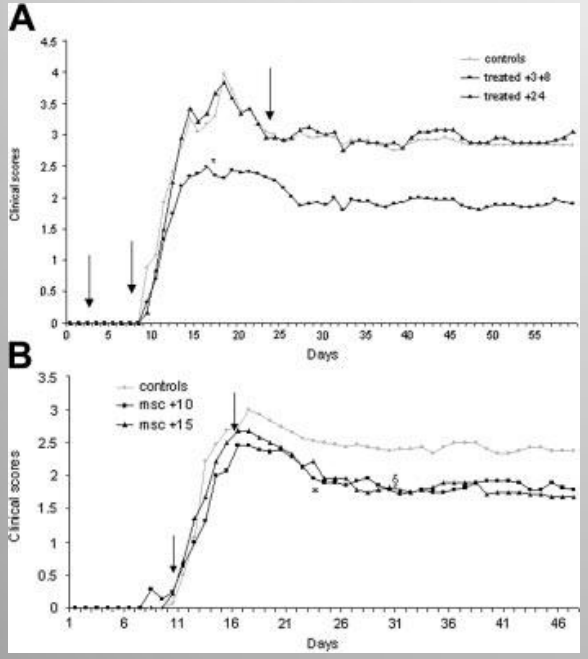
- **Karussis et al (Jerusalem) injected BMSC in 14 patients with ALS and 13 with MS, using both intravenous and lumbar intrathecal routes. Data was not published but they report no major adverse events and preliminary evidence of efficacy at 1 year follow-up (AAN,April 2010).**
- **In a recently published phase I trial from Bristol-UK, Scolding and colleagues injected 6 patients with advanced MS with bone marrow-derived stem cells and followed them for 1 year. There were no major adverse events with possible theraputic efficacy.**
- **Mazzini et al (Italy) transplanted surgically BMSC into the thoracic spinal cord of 10 ALS patients in a phase I trial. They reported no major adverse events but the downhill course of the disease was unchanged.**
- **A phase I trial of autologous BMSC stereotactically transplanted in the striatum of Parkinson patients is currently recruiting. Cells are expected to grow up into dopamine secreting neural cells.**

ZAPPIA

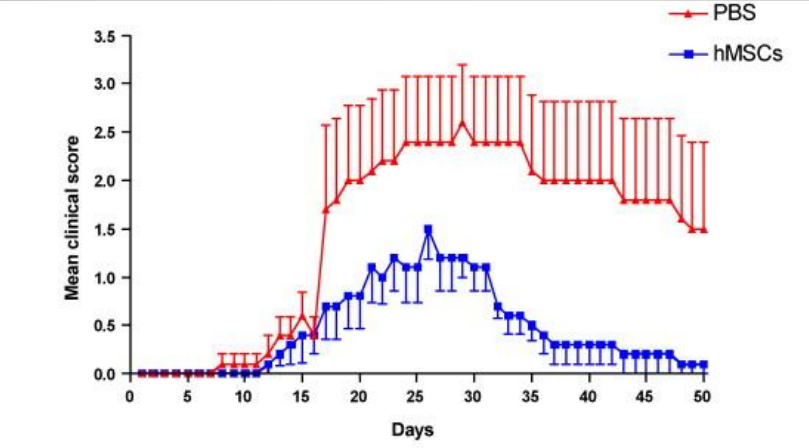




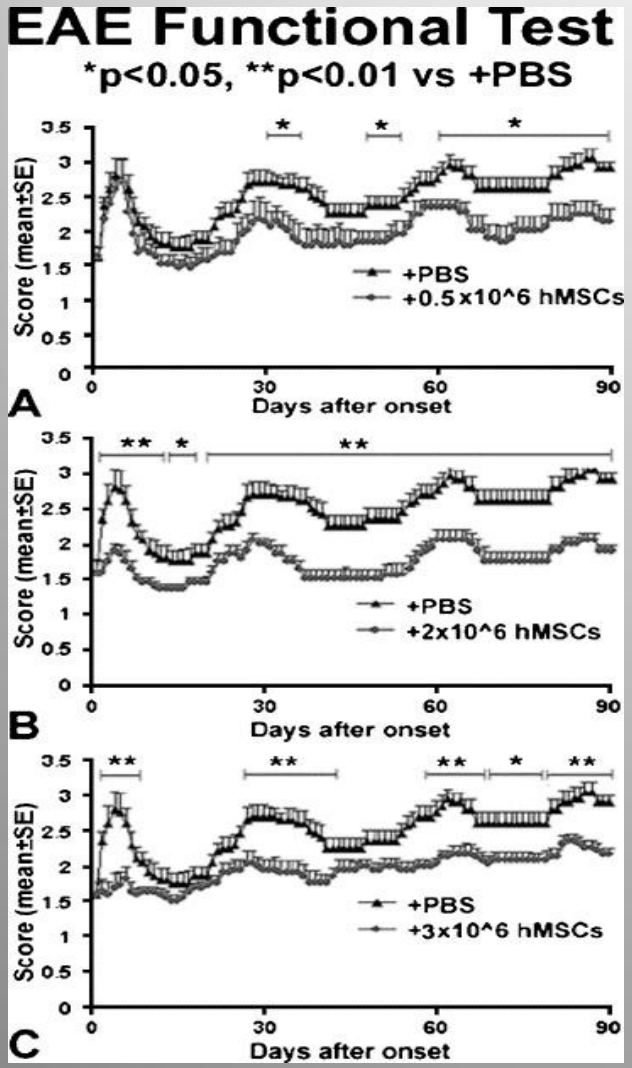
ZAPPIA


A
controls
treated +3+8
treated +24
Clinical scores
Days
B
controls
msc +10
msc +15
Clinical scores
Days

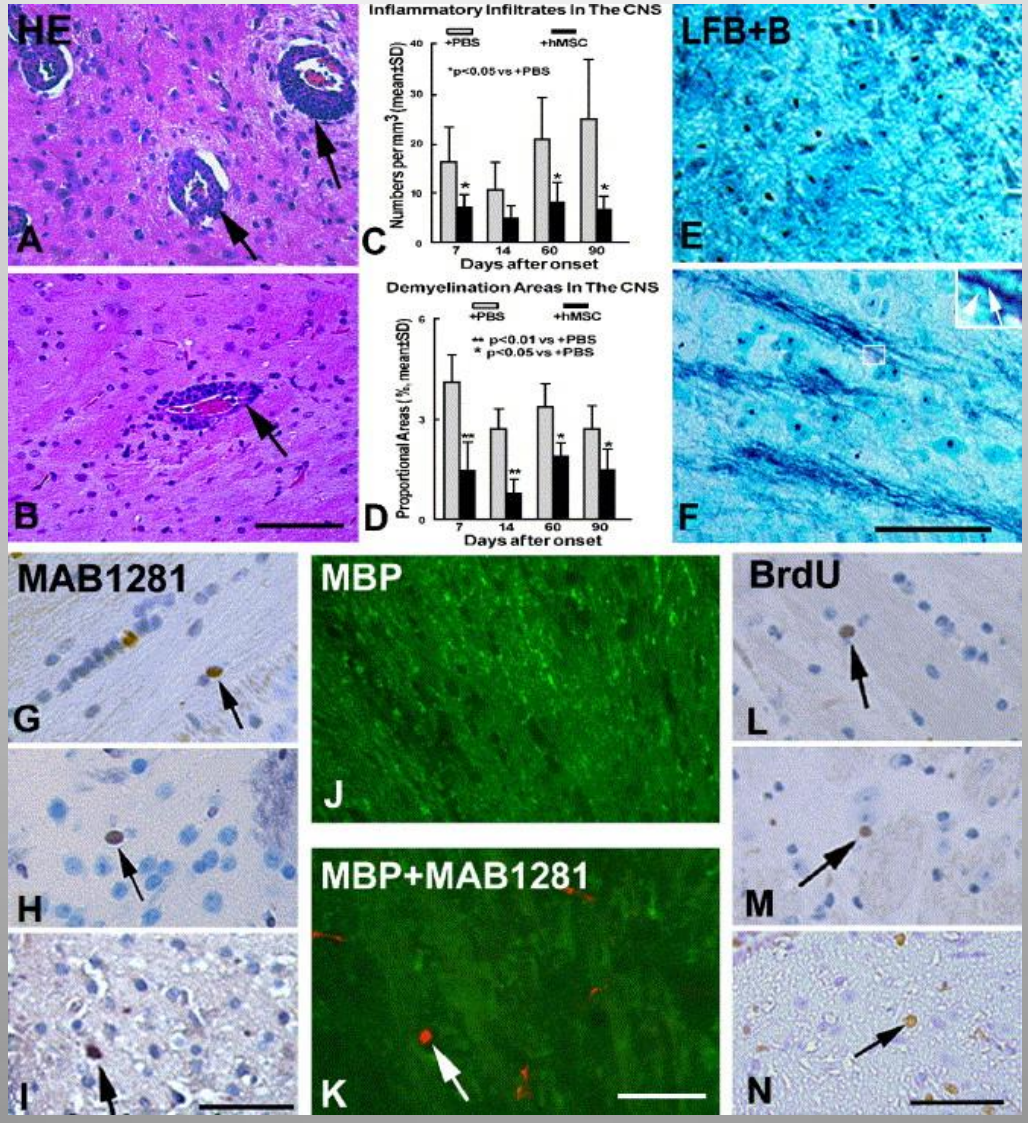
SCOLDING


PBS
hMSCs
Mean clinical score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
45
50
Days

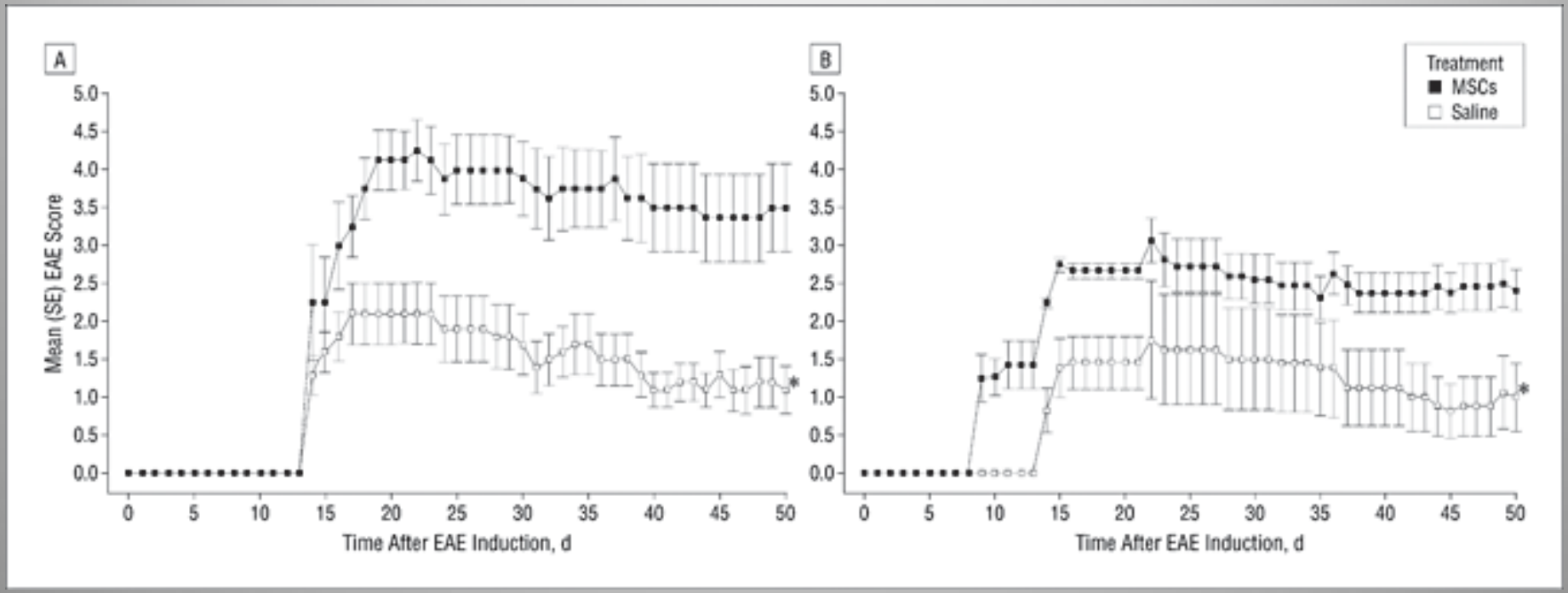
ZHANG


EAE Functional Test
*p<0.05, **p<0.01 vs +PBS
Score (mean±SE)
Days after onset
+PBS
+0.5x10^6 hMSCs
A
+PBS
+2x10^6 hMSCs
B
+PBS
+3x10^6 hMSCs
C

ZHANG


HE
A
B
Inflammatory Infiltrates In The CNS
+PBS
+hMSC
*p<0.05 vs +PBS
Numbers per mm3 (mean±SD)
C
Days after onset
Demyelination Areas In The CNS
+PBS
+hMSC
** p<0.01 vs +PBS
* p<0.05 vs +PBS
Proportional Areas (%, mean±SD)
D
Days after onset
LFB+B
E
F
MAB1281
G
H
I
MBP
J
MBP+MAB1281
K
BrdU
L
M
N

Clinical course of chronic experimental allergic encephalitis (EAE) in mice treated with saline (controls) or mesenchymal stromal cells (MSCs) 10 days after EAE induction (2 of 8 experiments)



Kassis, I. et al. Arch Neurol 2008;65:753-761.

ARCHIVES OF NEUROLOGY

Axonal Damage and Loss in Mice With Experimental Allergic Encephalitis (EAE) Treated With Mesenchymal Stromal Cells (MSCs)

Table 2. Axonal Damage and Loss in Mice With Experimental Allergic Encephalitis (EAE) Treated With Mesenchymal Stromal Cells (MSCs)

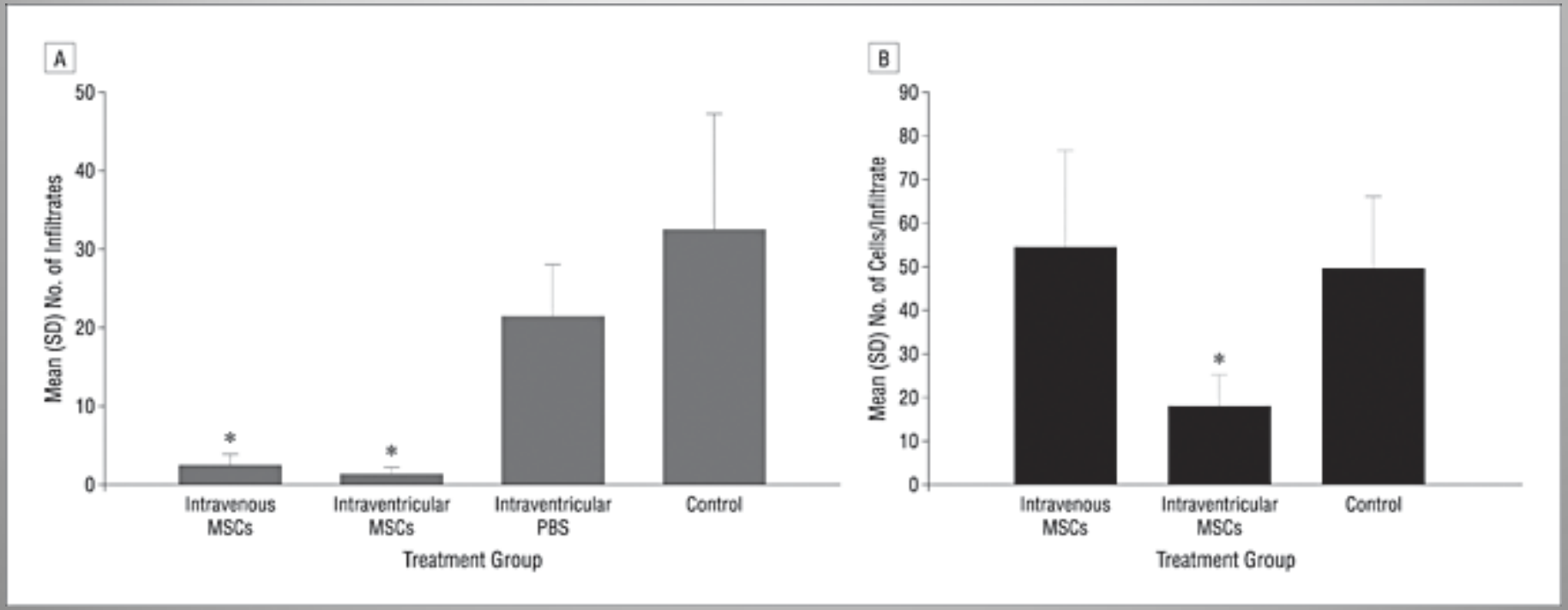
	Axonal Injury, Mean (SE), %					
Treatment Group	Normal	Few Scattered	Focused Mild to Moderate	Scattered Mild to Moderate or Focused Severe	Scattered Severe	Axonal Loss Score, Mean (SE)[a]
Control	40 (5)	26.7 (5)	10.0 (5)	20.0 (5)	3.3 (5)	1.75 (0.3)
Mice with EAE						
Intraventricular PBS	56.4 (5)	23.1 (5)	5.1 (5)	12.8 (5)	2.6 (5)	
Intravenous PBS	46.0 (5)	30.0 (5)	10.0 (5)	10.0 (5)	4.0 (5)	
Intraventricular MSCs	95.8 (5)[b]	4.2 (5)[b]	0	0	0	0.3 (0.15)
Intravenous MSCs	85.0 (5)[c]	10.0 (5)[c]	5.0 (5)	0	0	1.3 (0.4)

Abbreviation: PBS, phosphate-buffered saline.
[a] Axonal loss: 0 = normal axonal density; 1 = focused mild to moderate axonal loss; 2 = scattered mild to moderate axonal loss; 3 = focused severe axonal loss; and 4 = scattered severe axonal loss.
[b] For statistical comparisons, the χ^2 test for between-group comparison and the nonparametric Kruskal-Wallis test were applied, $P < .001$.
[c] For statistical comparisons, the χ^2 test for between-group comparison and the nonparametric Kruskal-Wallis test were applied, $P < .05$.

Kassis, I. et al. Arch Neurol 2008;65:753-761.

ARCHIVES OF NEUROLOGY

Lymphatic infiltration in the central nervous system in mice with chronic experimental allergic encephalitis treated with either saline (controls) or mesenchymal stromal cells (MSCs)



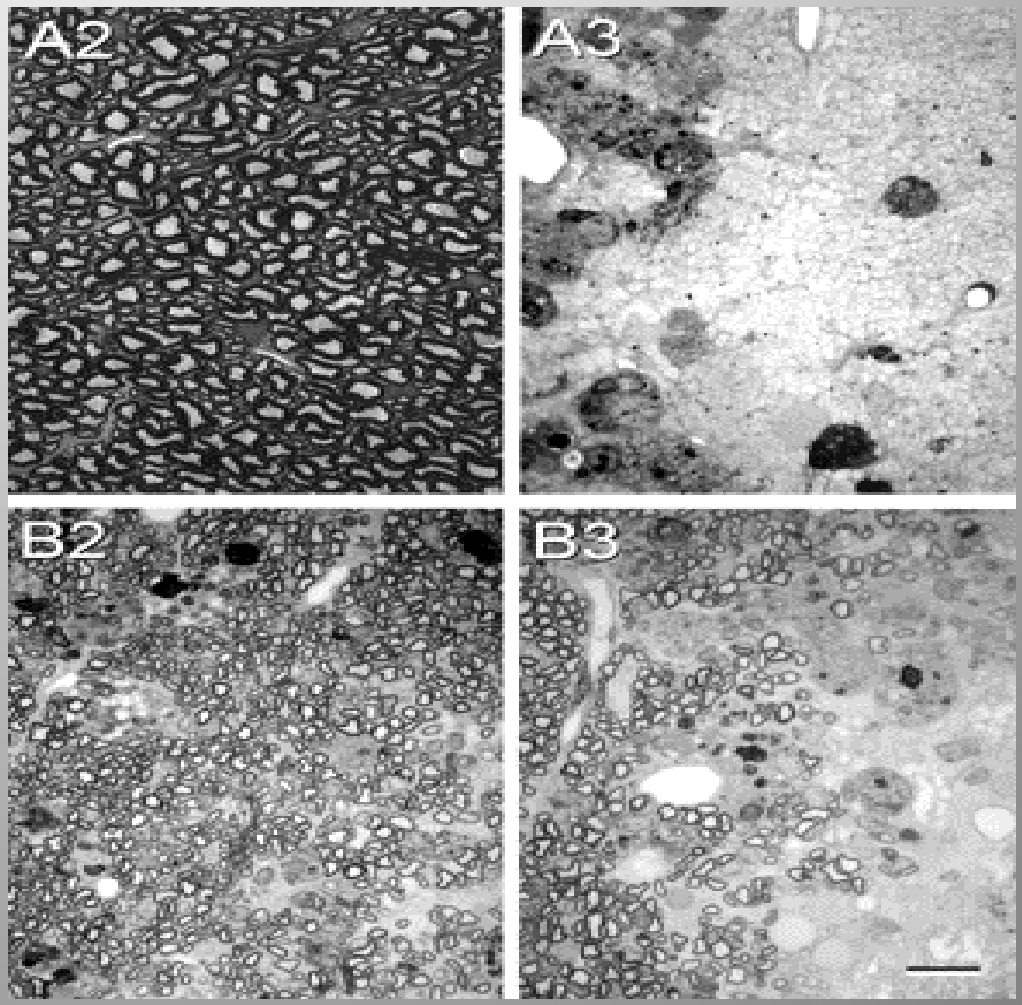
Kassis, I. et al. Arch Neurol 2008;65:753-761.

ARCHIVES OF NEUROLOGY

Animal Experiments

- **Experiments using BM mesenchymal stem cells in animal models with :**
  - **Spinal cord transection**
  - **EAE**
  - **Focal demyelination**

Gave promising results in terms of remyelination and clinical recovery



Akiyama et al, J Neurosc, August, 1, 2002