Beneficial effects of autologous mesenchymal stem cell (MSC) transplantation in progressive multiple sclerosis: Report of a randomized phase II double blind trial

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**Abstract**

**Background/Objective:** Mesenchymal stem cells (MSCs) induce immune-modulatory and neurotrophic effects and have an acceptable safety profile. This study evaluated the safety and efficacy of MSC transplantation in multiple sclerosis (MS).

**Methods:** This double-blind crossover trial (NCT02166021) enrolled 48 patients with active progressive MS (mean Expanded Disability Status Scale [EDSS] score: 5.6 0.8). Patients were randomized into three groups and treated intrathecally (IT) or intravenously (IV) with either autologous MSCs (1 × 106/kg) or sham injections (normal saline). After 6 months, half of the patients from the first two groups were re-treated with MSCs, and the other half, with sham injections. Patients from the third group, initially assigned to sham-treatment, were divided into two subgroups and then treated with either MSC-IT or MSC-IV.

**Results:** No serious, treatment-related adverse events were observed. Significantly fewer patients experienced neurological deterioration in the MSC-IT and MSC-IV groups compared with those in the sham-treated group (6.7%, 9.7%, and 48.4%, respectively, p = 0.0003 and p = 0.0008, chi-squared test). During the one-year duration of the study, 58.6% and 40.6% of patients treated with MSC-IT and MSC-IV, respectively, exhibited no evidence of disease activity (NEDA, i.e., no relapses, EDSS progression, nor activity on magnetic resonance imaging [MRI]), compared with 9.7% in the sham-treated groups (p < 0.0001 and p < 0.0048, respectively, chi-squared test). Treatment with MSC-IT induced significant benefits in monthly changes of the T2 lesion load on MRI, and the timed 25-foot walking test, 9-hole peg test, optical coherence tomography (OCT), and cognitive tests.

**Conclusions/Interpretation:** Autologous MSCs (IT and IV) were well-tolerated in progressive MS and induced robust benefits regarding all primary endpoints. The IT administration was more efficacious, indicating that the observed effects are possibly mediated by neurotrophic/neuroprotective and central immunomodulatory mechanisms. A phase III trial is warranted to confirm these findings.

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**1. Introduction**

Mesenchymal stem cells (MSCs) are non-hematopoietic stromal cells, which reside mainly in the bone marrow compartment, but also in fat and other tissues. Their classical role is to support hematopoiesis and produce cells of the mesodermal lineage 1,2. Studies have described additional MSC properties, including immunomodulatory and neurotrophic effects 3-12. In preclinical studies, intravenous (IV) and intrathecal (IT) administration of MSCs has been shown to suppress experimental autoimmune encephalomyelitis (EAE) 6,13,14 and support remyelination following spinal trauma or induced demyelination 15-17.

Few small, mostly open-label, clinical trials have reported indications of favorable effects of MSC treatment in stroke, multi-system atrophy, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) 18-29. Whether the observed benefits were mediated by immunomodulatory mechanisms or by neurotrophic and neuroprotective effects remains controversial.

These earlier studies prompted us to perform a controlled study to examine the therapeutic efficacy of MSC transplantation in progressive MS, for which there is a critical unmet need for treatment. We also aimed to investigate the most favorable route of cell delivery.

We present here the results of this double-blind trial that compared the clinical effects of IV or IT injections of autologous bone marrow-derived MSCs (1 × 106/kg) with those of sham injections, in 48 patients with progressive MS.

**2. Methods**

**2.1 Patients and study design**

The study (NIH registration: NCT02166021) was initiated in February 2015 and completed in June 2018. It was approved by the local ethics committee and Ministry of Health (MOH), and monitored by an external contract research organization (CRO) (BRD, Israel) and an external safety committee.

Over 200 patients from the Hadassah MS Centre and Unit of Neuroimmunology were pre-screened for inclusion in the trial. Patients meeting the following inclusion criteria, were enrolled: age ≤ 65 years; diagnosis of active and progressive MS (according to the 2013 revised criteria by Lublin et al. 30); treatment failure with at least one line of MS therapy; and Expanded Disability Status Scale (EDSS) score between 3.0 and 6.5. In total, 48 patients were included (20 female, 28 male), with a mean EDSS score at inclusion of 5.54 0.9, mean age of 47.3 9.3 years, and mean disease duration of 12.0 7.4 years (Figure 1-flow chart of the study, Table 1). The higher proportion of male vs. female patients in our sample (which is not in line with the gender distribution of MS) may be explained by the “biased” inclusion of only patients with progressive MS, who were non-responders to conventional MS treatments.

Thirty-nine patients had secondary progressive MS (SPMS), including 10 with superimposed relapses (“relapsing-progressive”), and nine had primary progressive MS (PPMS). Most patients (77%) had been previously treated with two or more of the accepted immunotherapeutic drugs for MS. All immunomodulatory treatments were stopped 3–6 months before the screening visit. Detailed demographic data of each patient are presented in supplementary Table 1. Patients gave written informed consent and were randomized by an external CRO (BRD, Israel) into three groups of 16 patients each, after stratification according to the EDSS scores. One group was assigned to receive an IT injection of 1 × 106 MSCs (MSC-IT)/kg of body weight, and an IV sham injection of normal saline (NS). The second group was treated with an IT sham injection (NS) and an IV injection of 1 × 106 MSCs (MSC-IV)/kg of body weight, and the third group (sham-treatment) was treated with NS (IV and IT). At baseline, the three groups showed no significant differences in EDSS score, gender, or disease duration (Table 1).

The patients were followed-up in the outpatient MS-clinic by the assigned “examining physicians” and underwent neurological examination (including EDSS/Functional Systems (FS) scoring), the 9-hole peg test, timed 25-foot walking test, MRI (including resting functional MRI [fMRI]), visual evoked potentials (VEP), optical coherence tomography (OCT), and cognitive, immunological, and visual dynamic tests at predetermined time-points, as displayed in the study flowchart (Figure 1). All physicians, clinic personnel, and patients were blinded to the treatment assignment. One patient (005) withdrew his consent 3 weeks after the first treatment. Compliance with the trial was excellent, and only nine out of the scheduled 528 visits were missed.

**2.2 MSC preparation and administration**

The MSCs were obtained from the bone marrow of each patient and prepared using a previously described protocol with slight modification 22 (see supplementary Methods section). The treating physician received two sealed syringes (covered with black adhesive), which were prepared by the laboratory investigator, according to the randomization number received from the CRO. The syringes contained either MSCs (1 × 106/kg of body weight) resuspended in 3 mL of NS or NS alone. The treating physician injected the full 3 mL of contents in the sealed syringe to the cerebrospinal fluid via lumbar puncture at the level of L4–5, using a 20-gauge needle and 3-way cannula. The 3 mL of the second syringe was injected into an aluminum-covered 500-mL sac with NS and infused to the patient over 30 min using a 20-gauge vein catheter. A volume of 3 mL of cerebrospinal fluid was removed for future testing.

**2.3** **Primary and secondary endpoints**

The two predetermined primary endpoints of the trial were: (a) the safety of the MSC-IV and -IT treatments (incidence of adverse events vs. those in the sham-treated group), and (b) the differences among the three groups in EDSS score changes and the proportion of patients with treatment failure, as evidenced by an increase in EDSS score or deterioration in any of the functional systems, at 6 and 12 months. Secondary endpoints included the differences between the sham-treated and the MSC-IT or MSC-IV treated groups in: (a) the number of relapses and the relapse rate; (b) the number of MRI gadolinium-enhancing lesions; (c) the annualized rate of change in the T2 lesion load on MRI, total normalized brain volume (percent brain volume change, PBVC), and fMRI-network connectivity strength vs. the rates during the run-in period; (d) the timed 25-foot walking and 9-hole peg tests; (e) cognitive functions; and (f) the retinal nerve fiber layer (pRNFL) thickness, and macular thickness and volume, as evaluated via OCT.

**2.4 Lesion load on MRI and brain volumetric changes**

For conventional 3T MRI, raw data were sent to the NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, Germany and evaluated in a blinded manner.

The methods of evaluation are described in the supplementary Methods section.

**2.5 fMRI, VEP, OCT, and cognitive tests**

Resting-state blood oxygenation level-dependent fMRI, VEP, OCT, and a battery of cognitive tests that are sensitive to MS were performed (including the Paced Auditory Serial Addition Test, PASAT; Brief Visuospatial Memory Test-Revised, BVMT-R; Symbol Digit Modalities Test, SDMT; Owatonna cognitive behavioral test, OWAT; KAVE-naming and fluency test, KAVE; Rey-Auditory Verbal Learning Test, RAVLT; and Trail Making Test, TMT), by standard techniques described in the supplementary Methods section.

**2.6 Statistical analysis**

The sizes of the experimental groups were calculated based on an expected treatment efficacy of at least 50% vs. sham-treatment to provide 80% power, assuming a standard deviation of differences of < 75%, using a paired *t*-test with a 0.050 two-sided significance level. The locked database was transferred from the CRO (BRD, Israel) to the external expertise company for medical statistical analysis (MedStat, Israel). All measured variables and derived parameters were assessed individually and tabulated using descriptive statistics. For categorical variables, summary tables were provided, giving the sample size and absolute and relative frequencies by study group. For continuous variables, summary tables were also provided, giving the sample size, arithmetic mean, standard deviation, median, minimum, and maximum by study group. Within-group changes from baseline or from the run-in period were analyzed using paired *t*-tests.

The two-sample non-parametric Wilcoxon–Mann–Whitney rank sum test was used to analyze the differences in quantitative parameters between the MSC-IT or MSC-IV and sham-treated groups. The chi-squared test was used to analyze the differences in binary parameters between the MSC-IT or MSC-IV and sham-treated groups. For fMRI, a single functional *z*-score reflecting network connectivity was calculated per scan for each subject.For cognitive functions, values were expressed as *z*-scores, which were calculated using normative data from the literature, adjusted for age, gender, and educational level. All tests were two-tailed, and results with a p-value of 0.05 or less were considered statistically significant. The data were analyzed using the SAS ® version 9.3 software (SAS Institute, Cary, NC, USA).

**3. Results**

**3.1 Primary endpoints**

**3.1.1 Safety**

Three serious adverse events occurred during the study resulting in patient hospitalization. Two were related to relapses of MS, and one was due to an upper respiratory infection (not related to the treatment) that was resolved after a course of antibiotics. No other serious adverse events were observed during the 14 months of the trial. The full list of adverse events is presented in Table 2.

**3.1.2 Clinical efficacy**

A per-protocol analysis of the predetermined primary efficacy endpoints, showed that the percentage of patients with treatment failure (any deterioration in EDSS score) was significantly lower in the MSC-IT and MSC-IV groups compared with the sham-treated group (6.7% and 9.7% vs. 48.4%, respectively, p = 0.0003 between MSC-IT and sham-treatment, p = 0.0008 between MSC-IV and sham-treatment, chi-squared test for pooled data from both 6-month periods of the trial). Among the sham-treated patients, 75% experienced a deterioration in at least one FS, and only 28% experienced similar deterioration in the MSC-IT and MSC-IV groups (p = 0,0002, chi-squared test) (Table 3).

The mean EDSS score deteriorated in the sham-treated group and was improved in the MSC-IT and MSC-IV groups during both treatment cycles (p = 0.0002 and p = 0.007, respectively, vs. sham-treatment; Mann–Whitney test) (Figure 2, Table 3). A total of 12 patients showed improvement in the MSC-IT group, 10 in the MSC-IV group, and only one in the sham-treated group.

The changes in ambulation scores and in the sum of all functional system scores (sFS), followed the same trend, strongly favoring MSC-IT and MSC-IV treatments over sham-treatment (Table 3). For the changes in EDSS and FS scores, the MSC-IT was superior to the MSC-IV treatment (Table 3). Repeated intrathecal treatment (MSC-IT at both treatment cycles) produced significantly better clinical outcomes at 12 months (Table 3). In the group of patients treated twice with MSC-IT, half had a confirmed disability improvement (CDI) at the end of the whole trial (1 year), verified by two consecutive examinations 3 months apart), and no one exhibited confirmed disability progression.

Fifteen out of 32 patients in the sham-treated groups experienced at least one relapse during both 6-month periods of the study, compared with only seven in the MSC-IV group and two out of 32 in the MSC-IT group (46.9%, 21.9%, and 6.3%, respectively, p = 0.0002 for MSC-IT, and p = 0.035 for MSC-IV vs. the sham-treatment) (Table 3 and Figure 2). The mean annual relapse rates in both cycles of treatment were 0.06 ± 0.25, 0.28 ± 0.57, and 0.56 ± 0.67 in the MSC-IT, MSC-IV, and sham-treated groups, respectively (p = 0.0005, for MSC-IT vs. sham-treatment; p = 0.052, for MSC-IV vs. sham-treatment, Wilcoxon test).

**3.2 Secondary endpoints**

**3.2.1 MRI**

The mean number of gadolinium-enhancing lesions per patient during the two cycles of treatment were: 0.55 ± 1.03 in the sham-treated group, 0.17 ± 0.47 in the MSC-IT group, and 0.97 ± 1.93 in the MSC-IV group (p = 0.062 for MSC-IT vs. sham-treatment; p = 0.90 for MSC-IV vs. sham-treatment; p = 0.077 for MSC-IT vs. MSC-IV, Wilcoxon test) (Figure 2 and Table 3). The mean monthly rate of the T2-fluid attenuated inversion recovery (T2-FLAIR) lesion volume change compared to the rate during the run-in period was −0.024 ± 0.053 in the pooled group of patients treated with MSC-IT; −0.016 ± 0.036 in the MSC-IV group; and 0.003 ± 0.029 in the sham-treated group (p = 0.029 for MSC-IT vs. sham-treatment; p = 0.123 for MSC-IV vs. sham-treatment) (Table 3).

**3.2.2 fMRI**

Testing of the motor networks revealed a significant annual increase in the mean *z*-score of the MSC-IT group (0.108 ± 1.06 and 0.156 ± 0.68 at 3 and 6 months, respectively; pooled data), and a decrease/deterioration in the sham-treated group (−0.504 ± 1.06 and −0.288 ± 0.61 at 3 and 6 months, respectively). The changes in the MSC-IV group were 0.036 ± 0.88 at 3 months and −0.06 ± 0.816 at 6 months (p = 0.0675 and p = 0.042 for MSC-IT vs. sham-treatment at 3 and 6 months, respectively; p = 0.031 and p = 0.077 for MSC-IV vs. sham-treatment at 3 and 6 months, respectively) (Figure 2 and Table 3).

3.2.3 No evidence of disease activity (NEDA)

Seventeen out of the 29 (58.6%) MSC-IT-treated patients (for whom there were data for all parameters of NEDA) were evaluated as NEDA-3 status during the two pooled 6-month treatment periods, as compared with 40.6% (13/32) in the MSC-IV group and 9.7% (3/31) in the sham-treated group, (p < 0.0001 for MSC-IT vs. sham-treatment and 0.0048 for MSC-IV vs. sham-treatment). The percentages of those evaluated as NEDA-432 status (i.e., including annual brain volume loss of < 0.4% according to MRI) were 44.8% in the MSC-IT, 28.1% in the MSC-IV, and 9.7% in the sham-treated groups (p = 0.005 for MSC-IT vs. sham-treatment and p = 0.12 for MSC-IV vs. sham-treatment) (Table 3).

**3.2.4 Other parameters**

Statistically significant benefits were observed in the MSC-IT group in the 25-foot timed walking test, 9-hole peg test, optical coherence tomography of the retinal nerve fiber layer OCT-RNFL, PASAT, and KAVE/OWAT cognitive tests. Trends of beneficial effects were also observed in VEP, the SDMT, and proportion of T-regulatory cells (increased) (Table 3).

**Discussion**

With an aim to evaluate the safety and clinical efficacy of autologous MSC transplantation in progressive MS, our study revealed positive results in all predefined primary endpoints. No serious, treatment-related adverse effects were observed and significantly fewer patients in the MSC-IT and MSC-IV groups experienced treatment failure, as compared with the sham-treated group (Table 3). Significant changes favoring MSC-IT treatment over sham-treatment were also observed in the rate of progression in EDSS scores, ambulation index, sum of functional scores, 25-foot timed walking test, 9-hole peg test, PASAT, and OWAT/KAVE cognitive tests, and the rate of change in T2 lesion load on MRI, as well as newer biomarkers, such as OCT (RNFL) and fMRI (motor network). Repeated IT injection of MSCs at month 6 significantly boosted the effects observed during the first cycle of treatment. Beneficial (but less significant) effects were also observed in the MSC-IV group. Overall, the robust effects of MSC transplantation on various parameters that reflect neurological dysfunction and MS activity, may indicate induction and/or enhancement of neuroprotective mechanisms. These benefits seem to be of particular clinical significance, as they were observed in patients with progressive active MS unresponsive to conventional immunotherapies, and for which no other proven treatment options exist.

Despite the development of highly efficient and more targeted immunotherapies, two major unmet needs in the treatment of MS still exist: 1. the need for treatment to suppress compartmentalized and meningeal inflammation in the central nervous system (CNS), which seems to drive tissue injury and progression of disability 31-35. These compartmentalized inflammatory and degenerative activities seem to be less responsive to the majority of immunomodulatory drugs, accounting for the poor efficacy of most MS therapies in progressive MS, with the exception of ocrelizumab36,37.

2. The need for a treatment that may substantially promote regeneration-remyelination. Generally, the CNS loses its capacity for efficient regeneration and remyelination over time. This is especially pronounced in chronic neuroinflammatory and neurodegenerative diseases such as MS, possibly due to an insufficiency of growth factors or defective mobilization of intrinsic CNS stem cells/oligodendrocyte progenitors 38-41.

Based on their well described properties38,41-44, stem cells may represent a “logical” treatment approach to achieve those unmet needs and induce neuroprotection and enhance endogenous remyelination. Moreover, stem cells are strong immunomodulators that may potentially downregulate the localized and compartmentalized inflammation upon their migration to the CNS 32-34.Several studies have shown that embryonic, neuronal, and other adult stem cells can induce beneficial clinicopathological effects in animal models of neurological diseases, including MS 6,13,14,45-49. The MSCs are commonly used for such therapies, as they have the following practical advantages for clinical use over other types of stem cells: 1. they can be easily cultured and expanded in large quantities; 2. they can be obtained from the patient, thus eliminating the need for a donor, the risk of rejection, or the need for chemotherapy; and 3. they seem to be safe and carry low risks of malignant transformation. During the last decade, MSC treatments have been applied to various neurological diseases in small or pilot open-label trials 18,19,22-27,29,50, with promising indications.

The putative mechanism of action of MSCs in neurological diseases is controversial. Some investigators claim that the most prominent effects are mediated through peripheral immunomodulation 7,10,11,40,47,51,52.We have long advocated that neuroprotective and neurotrophic mechanisms play the most crucial role, as supported by our findings in animal models and pilot trials of MS and ALS 22,26. We speculate that IT injection, which brings a higher proportion of the injected cells into close proximity with damaged areas of the CNS, may induce more robust effects than IV injection. In the current study, IT transplantation of MSCs was indeed shown to be superior to IV administration in several efficacy parameters (Table 3). If peripheral immunomodulation was the dominant mechanism, we would expect the opposite to be true. The improvements observed in OCT and motor networks on fMRI, and the trend toward increased brain volume seem to support the involvement of neurotrophic or even neuroregenerative mechanisms, induced or accelerated by the injected MSCs. In contrast, the less pronounced effects of the treatment on gadolinium-enhancing lesions on MRI, advocate against a major contribution of peripheral immunomodulatory mechanisms.

The strengths of our trial include: (1) the inclusion of patients with active progressive MS, for which existing immunotherapies are usually ineffective; (2) the double-blind design, making this the first randomized controlled trial comparing IT vs. IV methods of MSC-administration and single vs. repeated treatment; and (3) the robust clinical benefits observed in several disease activity parameters, including newer biomarkers, such as fMRI-network connectivity, OCT, and cognitive testing. The limitations of our study include the small number of patients in each group and the crossover design (in the second cycle), which may have introduced a “carry-over” effect from the first cycle of treatment.

In summary, our results provide clear signals of clinical efficacy and indications of neuroprotection, induced by the administration of autologous MSCs in patients with progressive MS. Our findings suggest the superiority of IT over IV administration, and repeated over single injections of the cells. These data may contribute to the design of future trials with cell therapies and the use of objective biomarkers for the evaluation of neurodegeneration and neuronal regeneration. A larger, phase III study is warranted to confirm these observations and further evaluate the therapeutic potential of cellular therapy in neuroinflammatory and neurodegenerative diseases such as MS.

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**Table 1**

# Demographics

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Gender** | **Age at inclusion** | **Disease course** | **EDSS increase at last year** | **EDSS at inclusion** | **EDSS at baseline** |
| **MSC-IT**  **(n=16)** | 9M  7F | 49.05±7.2 | 5 PPMS  11 SPMS  (2 with relapses) | 0.72±0.51 | 5.75±0.77 | 6.19±0.31 |
| **MSC-IV**  **(n=16)** | 6M  10F | 47.42±10.4 | 1 PPMS  15 SPMS  (2 with relapses) | 0.78±0.75 | 5.63±0.83 | 5.84±0.77 |
| **Sham-treatment**  **(n=16)** | 11M  5F | 45.89±10.9 | 3 PPMS  13 SPMS  (5 with relapses) | 0.69±0.57 | 5.44±1.05 | 5.66±1.08 |
| **p-value**  **(Kruskal–Wallis test)** | 0.312 | 0.566 | 0.480 | 0.641 | 0.819 | 0.583 |

**Table 2**

**Adverse Events**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Adverse event:** | No. | | | | Related to Procedure | Related to Treatment |
| Run-in  N=48 | MSC-IT  N=16+16 | MSC-IV  N=16+16 | Sham-treatment  N=16+16 |  |  |
| **No AE's** | - | 5 | 4 | 3 |  |  |
| **Headache** | 0 | 9 | 10 | 8 | Yes | No |
| **Back pain** | 1 | 2 | 2 | 3 | Yes | No |
| **Viral infection** | 3 | 0 | 2 | 1 | No | No |
| **Upper respiratory infection** | 1 | 1 | 0 | 0 | No | No |
| **Fever** | 1 | 1 | 1 | 2 |  |  |
| **Urinary tract infection** | 2 | 1 | 0 | 0 | No | No |
| **Sinusitis** | 0 | 2 | 0 | 0 | No | No |
| **Fall** | 1 | 1 | 2 | 1 | No | No |
| **Fracture (leg/ hand)** | 2 | 1 | 0 | 0 | No | No |
| **Dizziness** | 0 | 2 | 1 | 0 | No | No |
| **Hematoma** | 1 | 1 | 0 | 0 | No | No |
| **Nausea** | 0 | 0 | 2 | 0 | No | No |
| **Melanoma (in situ)** | 1 | 0 | 0 | 0 | No | No |
| **Scabies infection** | 0 | 1 | 0 | 0 | No | No |
| **Peripheral facial nerve palsy** | 0 | 1 | 0 | 0 | No | No |
| **Toothache** | 0 | 0 | 1 | 0 | No | No |
| **Anorexia** | 0 | 0 | 1 | 0 | No | No |
| **Gout** | 1 | 0 | 0 | 0 | No | No |
| **Facial rash** | 0 | 1 | 0 | 0 | No | No |
| **Cervical pain** | 0 | 0 | 1 | 0 | Possible | No |
| **Infection distal arm** | 0 | 0 | 0 | 1 | No | No |

Three serious adverse events occurred during the experiment resulting of the patients' hospitalization. Two of them were related to relapses of MS, and one was due to upper respiratory infection, that resolved after treatment with antibiotics.

**Table 3**

**Efficacy parameters analysis (pooled data from both cycles of treatment)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Primary endpoints** | **IT-MSC** | **IV-MSC** | **Sham-treatment** | **p-value MSC-IT vs. sham-treatment** | **p-value MSC-IV vs. sham-treatment** | **p-value MSC-IT vs. MSC-IV** |
| Treatment failure (increase in EDSS) at 6 months (pooled analysis of both cycles of treatment) | 6.7 % (2 of 30) | 9.7 % (3 of 31) | 48.4 % (15 of 31) | **0.0003** (chi-squared) | **0.0008** (chi-squared) | NS |
| Treatment failure (increase in at least one FS) at 6 months (pooled analysis of both cycles of treatment) | 28.1 % (9 of 32) | 28.1 % (9 of 32) | 75.0 % (24 of 32) | **0.0002** (chi-squared) | **0.0002** (chi-squared) | NS |
| Change in EDSS at 3M | -0.30.3  (median:-0.5) | -0.10.4  (median:0) | +0.20.4  (median:0) | **<.0001** | **0.0010** | 0.0624 |
| Change in EDSS at 6M | -0.20.3  (median:0) | -0.10.4  (median:0) | +0.30.4  (median:0) | **<.0001** | **0.0002** | 0.3280 |
| Change in ambulation score at 3M | -0.91.1  (median:-1) | -0.31.2  (median:0) | +1.01.2  (median:+1) | **<.0001** | 0.1938 | **0.0375** |
| Change in ambulation score at 6M | -0.81.2  (median:-1) | -0.41.1  (median:0) | +1.31.3  (median:1) | **0.0009** | 0.0938 | 0.1239 |
| Change in sum of functional scores at 3M | -2.82.4  (median:-3) | -1.52.1  (median:-1) | +0.51.7  (median:+1) | **<.0001** | **0.0009** | **0.0177** |
| Change in sum of functional scores at 6M | -2.92.4  (median:-3) | -1.42.3  (median:-1) | +0.82.2  (median:+1) | **<.0001** | **0.0006** | **0.0127** |
| Mean number of relapses per patient | 0.060.25 | 0.280.58 | 0.560.67 | **0.0005** | 0.052 | 0.074 |
| Proportion of patients relapse-free | 93.8 %  (n=32) | 78.1 %  (n=32) | 53.1 %  (n=32) | **0.001** | 0.1 |  |
| **Secondary endpoints** |  |  |  |  |  |  |
| 25-feet walking time % changes over 6M | -5.316.3 | -6.417.7 | +14.025.4 | **0.0017** | **0.0009** | 0.81 |
| 9-peg hole test % changes over 6M (dominant hand) | -3.010.1 | -2.15.1 | +0.58.9 | 0.129 | 0.300 | 0.43 |
| 9-peg hole test % changes over 6M (non-dominant hand) | -5.57.9 | -1.67.5 | +1.311.2 | **0.0136** | 0.391 | **0.043** |
| MRI: % monthly changes in flair T2 lesion volume at 6M | -0.0240.053  (median:-0.004) | -0.0160.036  (median:-0.004) | +0.0030.029  (median:-0.0) | **0.029** | 0.123 | 0.50 |
| MRI: mean number of gadolinium-enhancing lesions during the two cycles of treatment (pooled) | 0.17±0.47  (total number of lesions=9) | 0.97±1.93  (total number of lesions=49) | 0.55±1.03  (total number of lesions=30) | 0.0636 | 0.9086 | 0.0776 |
| MRI: total brain volume changes over 6M (in ml) | +6.8 (first cycle)  +7.8 (2nd cycle) | -10.5 (1st cycle)  +18.7 (2nd cycle) | -5.5 (1st cycle)  +1.0 (2nd cycle) | 0.14 (3M), 0.53 (6M) | 0.62 (3M), 0.37 (6M) | 0.02 (3M), 0.3 (6M) |
| PASAT cognitive test % change at 3M | +69.9204.4 | +37.8320.0 | -36.1143.7 | **0.0007** | 0.240 | **0.014** |
| PASAT cognitive test % change at 6M | +11.9166.2 | +129.5545.0 | -6.5203.2 | 0.327 | 0.939 | 0.334 |
| OWAT (KAVE) cognitive test change at 3M (Z-scores) | +0.4740.83 | +0.1220.80 | -0.2820.60 | **0.013** | 0.12 |  |
| SDMT cognitive test change at 3M (Z-scores) | +0.10.7 | -0.30.9 | -0.10.6 | 0.18 | 0.20 | **0.02** |
| OCT-RNFL (G) right eye % changes over 6M | -0.23.2  (median: 0) | +0.12.4  (median: 0) | -0.32.7  (median:-0.4) | 0.844 | 0.429 | 0.57 |
| OCT-RNFL (G) left eye % changes over 6M | +1.02.6 | +0.13.2 | -0.73.1 | **0.038** | 0.417 | 0.273 |
| % VEP latency changes at 6 months (left eye) | 0.46.6 | 0.76.3 | 3.615.9 | 0.36 | 0.26 |  |
| % VEP latency changes at 6 months (right eye) | 0.85.2 | -1.86.9 | 1.85.8 | 0.63 | **0.034** |  |
| fMRI annual changes in motor network (over 3 months) (Z-scores) | +0.108±1.06 | +0.036±0.88 | -0.504±1.06 | 0.0675 | **0.0312** | 0.74 |
| fMRI annual changes in motor network (over 6 months) (Z-scores) | +0.156±0.684 | -0.06±0.816 | -0.288±0.612 | **0.0425** | 0.0774 | 0.80 |
| Immunology: CD4+/CD25+ T regs % change at 6 months vs. baseline | 307.2487.5 | 249.5382.8 | 123.6281.9 | **0.05** | 0.11 | 0.62 |
| NEDA over 6M | 58.6 %  (17 of 29) | 40.6 %  (13 of 32) | 9.7 %  (3 of 31) | **<.0001** | **0.0048** | 0.160 |
| NEDA-4 (including <0.4 % annual change in total brain volume) | 44.8 %  (13 of 29) | 28.1 %  (9 of 32) | 9.7 %  (3 of 31) | **0.005** | **0.12** | 0.17 |

**Table 4**

**Comparison of last visit at 12 months vs. baseline between single and repeated MSC-IT or MSC-IV treatment**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **IT-MSC (x1) (n=8)** | **IT-MSC (x2) (n=8)** | **IV-MSC (x1) (n=8)** | **IV-MSC (x2) (n=8)** | **p-value of Comparison** |
| **Change from baseline to 12 months in EDSS** | 0.250±0.27 | -0.188±0.26 | 0.063±0.32 | -0.063±0.18 | IT: 0.0238  IV: 0.4965 |
| **Change from baseline to 12 months in ambulation score** | 1.250±1.16 | -1.250±1.28 | 1.000±1.31 | -0.250±0.89 | IT: 0.0045  IV: 0.0836 |
| **Change from baseline to 12 months in sum of functional systems** | 0.250±2.96 | -4.464±3.34 | -0.375±1.77 | -1.625±2.56 | IT: 0.0183  IV: 0.2263 |
| **Change from baseline to 12 months in 25-foot timed walking** | 5.288±7.74 | -5.936±8.35 | 4.519±5.69 | -1.169±8.12 | IT: 0.0128  IV: 0.0455 |

**Figures legends**

**Figure 1**

**Study design and flowchart**

**Figure 2**

**Beneficial effect of MSC treatment on the progression of MS as evidenced by the changes in EDSS.**

Number of patients in each treatment subgroup (MSC-IT, MSC-IC, and sham-treatment) who deteriorated in EDSS score or were stable or improved in EDSS, during each 3 or 6 months period (pooled data from the two cycles of treatment).

p < 0.0001 (3 months) and p = 0.0003 (6 months) for MSC-IT vs. sham-treatment (chi-squared)

p = 0.0085 (3 months), p = 0.0008 (6 months) for MSC-IV vs. sham-treatment (chi-squared)

**Figure 3**

**Slopes of progression in EDSS score in the six subgroups of the trial**

Follow-up of the mean EDSS score in each treatment subgroup during the run-in period and the two cycles of the study. A projection of the expected disease progression based on the run-in period, is presented in the graphs as a line that follows the run-in period.

1. Group 1A: treatment with MSC-IT in both first and second cycles.
2. Group 1B: treatment with MSC-IT in the first cycle and sham-treatment in the second.
3. Group 2A: treatment with MSC-IV in both first and second cycles.
4. Group 2B: treatment with MSC-IV in the first cycle and sham-treatment in the second.
5. Group 3A: treatment with sham-treatment in the first cycle and MSC-IT in the second.
6. Group 3B: treatment with sham-treatment in the first cycle and MSC-IV in the second.

**Figure 4**

**Treatment with MSC induces beneficial effects on the progression of MS, as evidenced by the changes in EDSS score**

Longitudinal follow-up of themean EDSS scores in each treatment subgroup (MSC-IT, MSC-IC, and sham-treatment), during the run-in pre-treatment period, and at 3 and 6 months following each cycle of treatment. Each 3- or 6-month period (pooled data from the two cycles of treatment) in the group of patients treated once with MSC vs. the group that received double treatment, during the 12-month duration of the study. Comparison was made between the EDSS scores at month 12 (visit 3) vs. the baseline value at visit 3.

p < 0.0001 (month 3) and p = 0.0003 (month 6) for IT vs. sham-treatment (chi-squared test).

p = 0.0085 (month 3), p = 0.0008 (month 6) for IV vs. sham-treatment (chi-squared test).

**Figure 5**

**Incidence of clinical relapses and gadolinium-enhancing lesions in MRI**

Number of relapses and gadolinium-enhancing lesions (presented as black dots for sham-treatment, black squares for MSC-IV, and triangles for MSC-IT) in each patient of the three treatment subgroups (MSC-IT, MSC-IV, and sham-treatment) during both cycles (two 6-month periods). (Pooled data from the two cycles of treatment).

Cyc-1 = first cycle

Cyc-2 = second cycle

p < 0.0005 for relapses in the MSC-IT group vs. sham-treatment.

p = 0.052 for relapses in the MSC-IV group vs. sham-treatment.

p < 0.0005 for relapses in the MSC-IT group vs. sham-treatment.

p = 0.052 for relapses in the MSC-IV group vs. sham-treatment.

Mean number of gadolinium-enhancing lesions on MRI in the two cycles of treatment pooled together: 0.17 ± 0.47 in the MSC-IT group, 0.97 ± 1.93 in the MSC-IV group, and 0.55 ± 1.03 in the sham-treated group (p = 0.062 for MSC-IT vs. sham-treatment, p = 0.90 for MSC-IV vs. sham-treatment, p = 0.077 for MSC-IT vs. MSC-IV, Wilcoxon test).

**Figure 6**

**MSC treatment induces beneficial effects on the motor network on fMRI**

Changes in mean *z*-values of the motor network on fMRI in the group of patients treated with MSC-IT or MSC-IV vs. the sham-treated group, during both cycles of the study.

An annual increase was observed in the mean z-score of the MSC-IT group (0.108 ± 1.06 and 0.156 ± 0.68 at 3 and 6 months, respectively; pooled data), and a deterioration in the sham-treated group (-0.504 ± 1.06 and -0.288 ± 0.61 at 3 and 6 months, respectively). The changes in the MSC-IV group were +0.036 ± 0.88 at 3 months, and -0.06 ± 0.816 at 6 months (p = 0.0675 and p = 0.042 for MSC-IT vs. sham-treatment at 3 and 6 months, respectively; p = 0.031 and p = 0.077 for MSC-IV vs. sham-treatment at 3 and 6 months, respectively).

**References**

1. Lennon DP, Caplan AI. Isolation of human marrow-derived mesenchymal stem cells. Exp Hematol 2006;34:1604-5.

2. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.

3. Blondheim NR, Levy YS, Ben-Zur T, et al. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. Stem Cells Dev 2006;15:141-64.

4. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641-50.

5. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006;98:1076-84.

6. Kassis I, Grigoriadis N, Gowda-Kurkalli B, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. Arch Neurol 2008;65:753-61.

7. Kassis I, Vaknin-Dembinsky A, Karussis D. Bone marrow mesenchymal stem cells: agents of immunomodulation and neuroprotection. Curr Stem Cell Res Ther 2011;6:63-8.

8. Kassis I, Zangi L, Rivkin R, et al. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant 2006;37:967-76.

9. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-5.

10. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008;8:726-36.

11. Uccelli A, Pistoia V, Moretta L. Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol 2007;28:219-26.

12. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61:364-70.

13. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106:1755-61.

14. Harris VK, Yan QJ, Vyshkina T, Sahabi S, Liu X, Sadiq SA. Clinical and pathological effects of intrathecal injection of mesenchymal stem cell-derived neural progenitors in an experimental model of multiple sclerosis. J Neurol Sci 2012;313:167-77.

15. Cizkova D, Rosocha J, Vanicky I, Jergova S, Cizek M. Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. Cell Mol Neurobiol 2006;26:1167-80.

16. Hedayatpour A, Ragerdi I, Pasbakhsh P, et al. Promotion of remyelination by adipose mesenchymal stem cell transplantation in a cuprizone model of multiple sclerosis. Cell J 2013;15:142-51.

17. Zhang XM, Du F, Yang D, et al. Transplanted bone marrow stem cells relocate to infarct penumbra and co-express endogenous proliferative and immature neuronal markers in a mouse model of ischemic cerebral stroke. BMC Neurosci 2010;11:138.

18. Connick P, Kolappan M, Crawley C, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. Lancet Neurol 2012;11:150-6.

19. Fernandez O, Izquierdo G, Fernandez V, et al. Adipose-derived mesenchymal stem cells (AdMSC) for the treatment of secondary-progressive multiple sclerosis: A triple blinded, placebo controlled, randomized phase I/II safety and feasibility study. PLoS One 2018;13:e0195891.

20. Harris VK, Stark J, Vyshkina T, et al. Phase I Trial of Intrathecal Mesenchymal Stem Cell-derived Neural Progenitors in Progressive Multiple Sclerosis. EBioMedicine 2018;29:23-30.

21. Harris VK, Vyshkina T, Sadiq SA. Clinical safety of intrathecal administration of mesenchymal stromal cell-derived neural progenitors in multiple sclerosis. Cytotherapy 2016;18:1476-82.

22. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 2010;67:1187-94.

23. Lee PH, Lee JE, Kim HS, et al. A randomized trial of mesenchymal stem cells in multiple system atrophy. Ann Neurol 2012;72:32-40.

24. Llufriu S, Sepulveda M, Blanco Y, et al. Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. PLoS One 2014;9:e113936.

25. Lublin FD, Bowen JD, Huddlestone J, et al. Human placenta-derived cells (PDA-001) for the treatment of adults with multiple sclerosis: a randomized, placebo-controlled, multiple-dose study. Mult Scler Relat Disord 2014;3:696-704.

26. Petrou P, Gothelf Y, Argov Z, et al. Safety and Clinical Effects of Mesenchymal Stem Cells Secreting Neurotrophic Factor Transplantation in Patients With Amyotrophic Lateral Sclerosis: Results of Phase 1/2 and 2a Clinical Trials. JAMA Neurol 2016;73:337-44.

27. Planchon SM, Lingas KT, Reese Koc J, et al. Feasibility of mesenchymal stem cell culture expansion for a phase I clinical trial in multiple sclerosis. Mult Scler J Exp Transl Clin 2018;4:2055217318765288.

28. Riordan NH, Morales I, Fernandez G, et al. Clinical feasibility of umbilical cord tissue-derived mesenchymal stem cells in the treatment of multiple sclerosis. J Transl Med 2018;16:57.

29. Yamout B, Hourani R, Salti H, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol 2010;227:185-9.

30. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 2014;83:278-86.

31. Eshaghi A, Prados F, Brownlee WJ, et al. Deep gray matter volume loss drives disability worsening in multiple sclerosis. Ann Neurol 2018;83:210-22.

32. Lucchinetti CF, Popescu BF, Bunyan RF, et al. Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011;365:2188-97.

33. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089-104.

34. Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. N Engl J Med 2018;378:169-80.

35. Ruggieri S, Petracca M, Miller A, et al. Association of Deep Gray Matter Damage With Cortical and Spinal Cord Degeneration in Primary Progressive Multiple Sclerosis. JAMA Neurol 2015;72:1466-74.

36. Montalban X, Hauser SL, Kappos L, et al. Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med 2017;376:209-20.

37. Shivani A, Okuda DT, Stuve O. Therapeutic Advances and Future Prospects in Progressive Forms of Multiple Sclerosis. Neurotherapeutics 2016;13:58-69.

38. Ben-Hur T, Einstein O, Bulte JW. Stem cell therapy for myelin diseases. Curr Drug Targets 2005;6:3-19.

39. Einstein O, Ben-Hur T. The changing face of neural stem cell therapy in neurologic diseases. Arch Neurol 2008;65:452-6.

40. Karussis D, Kassis I. Use of stem cells for the treatment of multiple sclerosis. Expert Rev Neurother 2007;7:1189-201.

41. Karussis D, Petrou P, Kassis I. Clinical experience with stem cells and other cell therapies in neurological diseases. J Neurol Sci 2013;324:1-9.

42. Freedman MS, Bar-Or A, Atkins HL, et al. 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;16:503-10.

43. Freedman MS, Uccelli A. Neurorepair with mesenchymal stem cells: hope or hype? Lancet Neurol 2012;11:123-5.

44. Scolding NJ, Pasquini M, Reingold SC, et al. Cell-based therapeutic strategies for multiple sclerosis. Brain 2017;140:2776-96.

45. Aharonowiz M, Einstein O, Fainstein N, Lassmann H, Reubinoff B, Ben-Hur T. Neuroprotective effect of transplanted human embryonic stem cell-derived neural precursors in an animal model of multiple sclerosis. PLoS One 2008;3:e3145.

46. Ben-Hur T, Idelson M, Khaner H, et al. Transplantation of human embryonic stem cell-derived neural progenitors improves behavioral deficit in Parkinsonian rats. Stem Cells 2004;22:1246-55.

47. Karussis D, Kassis I. The potential use of stem cells in multiple sclerosis: an overview of the preclinical experience. Clin Neurol Neurosurg 2008;110:889-96.

48. Pluchino S, Gritti A, Blezer E, et al. Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. Ann Neurol 2009;66:343-54.

49. Pluchino S, Quattrini A, Brambilla E, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 2003;422:688-94.

50. Steinberg GK, Kondziolka D, Wechsler LR, et al. Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke: A Phase 1/2a Study. Stroke 2016;47:1817-24.

51. Karussis D, Kassis I, Kurkalli BG, Slavin S. Immunomodulation and neuroprotection with mesenchymal bone marrow stem cells (MSCs): a proposed treatment for multiple sclerosis and other neuroimmunological/neurodegenerative diseases. J Neurol Sci 2008;265:131-5.

52. Uccelli A, Morando S, Bonanno S, Bonanni I, Leonardi A, Mancardi G. Mesenchymal stem cells for multiple sclerosis: does neural differentiation really matter? Curr Stem Cell Res Ther 2011;6:69-72.