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# PILOT TRIAL OF INTRAVENOUS AUTOLOGOUS CULTURE-EXPANDED MESENCHYMAL STEM CELL TRANSPLANTATION IN MULTIPLE SCLEROSIS

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**Declaration of Conflicting Interests**

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## Abstract

**Background—**Mesenchymal stem cells (MSCs) exhibit immunomodulatory, tissue-protective, and repair-promoting properties in vitro and in animals. Clinical trials in several human conditions support the safety and efficacy of MSC transplantation. Published experience in multiple sclerosis (MS) is modest.

**Objective—**To assess feasibility, safety, and tolerability, and explore efficacy of autologous MSC transplantation in MS.

**Methods—**Participants with relapsing remitting (RR) or secondary progressive (SP) MS, Expanded Disability Status Scale score 3.0–6.5, disease activity or progression in the prior two years, and optic nerve involvement were enrolled. Bone-marrow-derived MSCs were culture-expanded, then cryopreserved. After confirming fulfillment of release criteria, 1–2 × $10^6$ MSCs/kg were thawed and administered IV.

**Results—**Twenty four of 26 screened patients were infused: 16 women and 8 men, 10 RRMS and 14 SPMS, mean age 46.5, mean Expanded Disability Status Scale score 5.2, 25% with gadolinium-enhancing MRI lesions. Mean cell dosage (requiring 1–3 passages) was 1.9 × $10^6$ MSCs/kg (range 1.3–2.0) with post thaw viability uniformly ≥95%. Cell infusion was tolerated well without treatment-related severe or serious adverse events, or evidence of disease activation.

**Conclusion—**Autologous MSC transplantation in MS appears feasible, safe, and well tolerated. Future trials to assess efficacy more definitively are warranted.

### Keywords

multiple sclerosis; clinical trial; mesenchymal stem cells; safety; disability measures; quantitative MRI

## Introduction

Multiple sclerosis (MS) pathology is characterized by multifocal central nervous system lesions with perivenular inflammatory cell infiltrates, demyelination, axonal transection, neuronal degeneration, and gliosis. Currently approved therapies are effective in relapsing MS, where they prevent accumulation of focal inflammatory damage, but none has been shown to directly promote repair.

Mesenchymal stem cells (MSCs), also known as multipotent stromal cells, can be isolated from multiple adult tissues including bone marrow, culture-expanded ex vivo, and induced to differentiate into cells of the mesodermal lineage, including osteoblasts, adipocytes, and chondrocytes. Their pleotropic immunomodulatory and repair-promoting effects and ability to traffic to areas of tissue inflammation and damage have led to testing in various inflammatory and tissue injury conditions, and also make them attractive as a potential treatment for MS.[1] Pilot studies utilizing cells from several sources and tested in a variety of clinical settings have supported their safety and potential efficacy.[1] An uncontrolled phase 1/2 study in secondary progressive (SP) MS suggested tissue repair within the afferent visual system.[2] A recent small randomized phase 2 crossover study in relapsing remitting (RR) MS suggested reduced gadolinium-enhancing brain MRI lesions.[3] Based on regulatory input on study design, we undertook a phase 1 study of autologous, culture expanded MSC transplantation in MS, focusing on feasibility, safety, and tolerability.

## Materials and Methods

### Study design

This open-label, phase 1 pre-post comparison study enrolled participants at the Cleveland Clinic Mellen Center for MS Treatment and Research between March 2011 and April 2013. Bone marrow aspiration was performed in the Dahms Clinical Research Unit at University Hospitals Cleveland Medical Center (UHCMC). MSC isolation and culture-expansion were performed in the National Center for Regenerative Medicine Cellular Therapy Laboratory at Case Western Reserve University, as previously described.[4] The Cleveland Clinic and UHCMC Institutional Review Boards approved the study. Participants gave written consent before any study-related procedures were performed. The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice[5] and the principles of the Declaration of Helsinki.[6] A Steering Committee designed the study and monitored its conduct. A Medical Monitor and independent Data and Safety Monitoring Committee reviewed trial conduct and safety data. Additional administrative details are available in the Supplementary Appendix.

## Participants

Eligible participants were 18–55 years of age and had RR or SP MS fulfilling the 2010 McDonald Criteria;[7] Expanded Disability Status Scale (EDSS) score 3.0–6.5;[8] documented relapse, worsening disability, or MRI lesion activity in the prior two years; evidence of optic nerve involvement clinically, on visual evoked potentials (VEP), or optic coherence tomography (OCT); and brain MRI demonstrating T2-hyperintense lesions fulfilling MS diagnostic criteria.[9] Participants could continue interferon-beta or glatiramer acetate, but other disease therapies were discontinued for varying durations prior to screening.

## Intervention

After completing required screening, eligible participants underwent posterior iliac crest bone marrow aspiration under local anesthetic to obtain 20–50 ml of marrow. Bilateral aspiration was performed when necessary. MSCs were isolated and culture-expanded using established standardized procedures.[4] At each passage after the primary culture, $1 \times 10^6$ cells were frozen for laboratory analysis. The cells were propagated in culture for up to eight weeks in low glucose DMEM containing 10% fetal bovine serum (FBS) prescreened for optimal MSC growth and 10 ng/ml human fibroblast growth factor-2 (R&D Systems, Minneapolis, MN). Conditioned culture medium was tested for microbiological contamination at each passage, and the culture terminated if microorganisms were detected. On the day of MSC harvest, the cells were detached from culture flasks using porcine trypsin and washed three times with PlasmaLyte plus 1% human serum albumin. Cell aliquots were evaluated for viability by trypan blue exclusion and cell surface phenotype by cytofluorometry with monoclonal antibodies for CD105, CD73, CD14, and CD45. An aliquot of the final cell product was tested for the presence of mycoplasma, and the conditioned medium was tested for aerobic and anaerobic bacteria, endotoxin, and fungus. MSCs were cryopreserved in a single Cryocyte freezing bag (plus two 1-ml satellite vials for quality assurance/quality control testing if needed), at final concentration of $4 \times 10^6$/ml, in PlasmaLyte A containing 10% dimethyl sulfoxide and 5% human serum albumin.

Release criteria for the post-expansion/pre-cryopreservation MSC product included:

- Availability of at least $1 \times 10^6$ MSCs/kg participant body weight.
- ≥90% positivity for CD105/CD73, and <5% positivity for CD14/CD45 surface markers.
- Absence of detectable bacteria, fungus, mycoplasma, or endotoxin.

In addition, ≥70% viability by trypan blue exclusion of an aliquot of the infusion product after thaw was required. If a culture was contaminated or provided inadequate cell yield, a second aspirate was requested from the participant.

After completion of the baseline (month 0) visit, participants received a single intravenous (IV) infusion of $1–2 \times 10^6$/Kg body weight MSCs without premedication. Vital signs, percutaneous oxygen saturation, electrocardiogram, and adverse events (AEs) were monitored for six hours following infusion.

## Procedures

Safety assessments (AE monitoring, concomitant medication review, vital signs, weight, height, percutaneous oxygen saturation by pulse oximetry, general physical examination, blood chemistry, complete blood count, and urinalysis) were performed at screening (median 2.7 months); baseline (month 0); post infusion days 1, 4, 7, 14, 21; and months 1, 2, 3, 6. Clinical immunology studies (thyroid stimulating hormone, anti-thyroglobulin antibodies, anti microsomal antibodies, sedimentation rate, C-reactive protein, anti nuclear antibodies, SSA, SSB, rheumatoid factor, and lymphocyte subsets by cytofluorometry) were performed at screening; baseline; and months 1, 3, and 6. Chest X-ray and electrocardiogram were performed at screening, and months 1 and 6. Participant self reported global well being using a visual analog scale, neurological examination and calculation of EDSS, Multiple Sclerosis Functional Composite (MSFC), high and low-contrast letter acuity using Sloan charts (LCLA), MRI, VEP, and OCT were performed at screening; month-1; baseline; and months 1, 2, 3, and 6. MRI scans were acquired on a 3T Siemens Trio MRI scanner (Erlangen, Germany) according to a standardized protocol (see Supplementary Appendix) and analyzed by the MRI Analysis Center (Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, OH). OCT was performed using a Cirrus spectral domain OCT machine (Carl Zeiss Meditec, Dublin, CA).

## Outcomes

The study's primary objective was to evaluate the feasibility of culturing MSCs, and infusion-related safety and tolerability of autologous MSC transplantation over one month in participants with relapsing forms of MS. Secondary objectives included evaluation of safety and tolerability over six months and effects on disease activity measured by the number of gadolinium-enhancing MRI lesions at one month. Safety measures included AEs, vital signs, oxygen saturation, blood laboratory studies, clinical immunology studies, urinalysis, chest X-ray, and electrocardiogram. AE severity was categorized based on Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Pre-specified exploratory efficacy measures included participant self report of global well being using a visual analog scale; MS disease status (relapses, EDSS, MSFC); vision (high-contrast visual acuity, LCLA); brain MRI (gadolinium enhancing lesion number and volume, new/enlarged T2-hyperintense lesion number, T2-hyperintense and T1-hypointense lesion volumes, normalized whole brain volume [brain parenchymal fraction], gray matter fraction, whole brain diffusion tensor imaging [mean diffusivity, fractional anisotropy], whole brain magnetization transfer ratio [MTR]); VEP (P100 latency); and OCT (retinal nerve fiber layer thickness, foveal thickness, macular volume).

MS relapse was defined as new, recurrent, or increased MS symptom(s); developing acutely (evolving over less than 3 months); with onset at least 30 days after the onset of a previous confirmed relapse; lasting at least 24 hours; with new objective neurological findings on the examination; and not associated with infection, fever, increased body temperature for another reason, or metabolic derangement. Confirmed relapses could be treated at the Treating Neurologist's discretion with IV methylprednisolone 1000 mg/day for 3–5 days without an oral taper.

### Statistical methods

Clinically significant treatment-related AEs were expected to be infrequent (<5% of participants), based on accumulating experience with MSC transplantation.[10] The trial was conducted under a group sequential monitoring plan, with interim examinations of treatment related AEs after every fourth participant was followed for one month after infusion. Early stopping was to be considered if three participants within the first eight, or four participants overall, experienced treatment related AE CTCAE grade ≥3 or treatment related serious AE at any point during follow-up. This procedure followed an O'Brien-Fleming stopping rule for a nominal $\alpha$=5% one-sided test of the composite null hypothesis of a 5% or lower risk of such a treatment associated AE against the alternative of a higher risk. This plan gives a ≤3.2% chance of a false finding of harm when true risk is <5%, and 89%, 74%, 50%, and 22% power to detect true risks of 25%, 20%, 15%, and 10%, respectively.

Descriptive methods were chosen appropriate to each variable's scale (i.e., nominal, ordinal, interval, ratio). Exploratory objectives were to assess the effect of MSC transplantation on the longitudinal course of MS. To this purpose, we fit mixed linear statistical models with random subject effects and, wherever possible, a time series covariance structure, to numerous continuous MS outcome measures, and examined trends using restricted cubic splines and custom tests between specified inter-visit comparisons. Categorical data and nonparametric methods were used for outcomes for which such models were inappropriate. As this was an open label pilot study without a parallel control arm, and not powered to reliably detect temporal trends due to efficacy, p-values were used to describe compatibility of the data with absence of MSC activity, but not for formal hypothesis testing. For most outcomes, the primary exploratory p-value compared slopes or means within three months post-infusion with the 2–3 month pre-infusion period.

## Results

Of 24 participants initially consented, one exhibited gadolinium allergy during the screening MRI and was not enrolled. Another's culture failed due to slow growth and development of atypical cellular morphology, first noted at the first passage. The participant had no distinguishing past medical history, MS features, or previous or concomitant medications. The participant elected not to undergo a second bone marrow aspiration and discontinued the study. Two replacement participants were consented and enrolled (Figure 1). Demographic, clinical, and imaging characteristics for the 25 enrolled participants are summarized in Table 1. Among the infused participants, yields following the initial Percoll gradient centrifugation, final yields, and kinetics of growth varied considerably, with no apparent relation to participant demographics, MS disease characteristics, or current or prior treatment, and despite adherence to standard protocols for bone marrow harvest and cell culture. Nevertheless, the cell dose was within the target range for all infused participants and for 21 (88%) within 5.5% of $2 \times 10^6$/kg (Table 2). All planned visits and assessments were completed except for one blood study for one participant.

There were no severe (CTCAE grade ≥3) or serious AEs related to study treatment. Table 3 lists AEs experienced by ≥2 participants from infusion through follow-up. One participant with SP MS died eight months after completing the study. After study follow-up, this patient

had progressive neurologic disability, with further worsening after intrathecal baclofen pump placement requiring two revisions, and died in hospice with severe spastic quadriplegia but no unusual infections or other medical condition. A second participant with SP MS died 40 months after completing the study after choking on food. We are unaware of other participants with unexpected AEs after study completion.

No participant developed clinical manifestations or serologic studies indicating autoimmune phenomena. There was no clinical or radiographic evidence of paradoxical MS disease activation (Table 4). Although an active MRI was not required at enrollment, 24–50% of MRIs at the three pre infusion time points demonstrated gadolinium enhancing-lesions. Post-infusion, there was no evidence of activation or inhibition of gadolinium enhancement or new/enlarged T2 hyperintense MRI lesion accumulation. Among 24 infused participants, 18 (75%) were free of relapse, three (12.5%) experienced a single relapse during post-infusion follow-up, and three (12.5%) experienced two relapses (two pre-infusion, four post infusion). All relapses but one occurred in participants with RR MS. The single relapse in a participant with SP MS (but with a gadolinium-enhancing lesion at screening) was documented at the final visit. Among participants with RR MS, annualized relapse rates were 0.81 (95% CI 0.10–2.93) pre-infusion, 1.85 (95% CI 0.50–4.73) in months 1–3 post infusion, and 0.90 (95% CI 0.11–3.24) in months 4–6 post-infusion (p=0.58).

Most other exploratory efficacy outcomes were generally stable over the course of the study (Table 4). Descriptive p values fell below the conventional 5% for three outcomes, compatible with two expected by chance alone based on number of outcomes studied. These outcomes were whole brain MTR (p=0.03) and MTR peak height (p=0.008), both of which trended upwards in post infusion months 1–3 relative to pre-infusion, and EDSS, for which three post-infusion measurements worsened while 17 were improved from baseline (signed rank p=0.001), with others stable. Improvement in the EDSS reflected improvements in ambulation, and cerebral and sensory subsystems.

## Discussion

Previous experience in other disorders and limited published experience in MS suggested MSC transplantation would be safe and well tolerated. However, unanticipated autoimmune AEs have occurred with other MS therapies, e.g. alemtuzumab,[11] altered peptide ligands,[12] and tumor necrosis factor blockade.[13] Therefore, based on regulatory input on study design, we conducted a pilot study focusing on feasibility, safety, and tolerability. During our study's start-up preparations, the MSCT Study Group recommended a phase 2 design,[14] stimulating the multinational Mesenchymal Stem Cells for Multiple Sclerosis (MESEMS) trial from which preliminary results recently were reported by a participating center.[3]

Our phase I study results support the feasibility, safety, and tolerability of IV administration of autologous, culture-expanded, bone marrow derived MSCs in MS. No treatment-related severe or serious AEs occurred during the follow-up period. The deaths of two participants after completing the trial did not appear to be related to MSC transplantation. Since the study was relatively small with only six-month follow up, we cannot rule out the possibility of rare or late-appearing AEs.

Exploratory efficacy measures demonstrated no substantial evidence of inhibition of disease activity, tissue repair, or recovery of function comparing 2–3 months pre infusion to six months post-infusion. Given the lack of comparison group, small sample size, and relatively short follow-up, demonstration of benefit was not anticipated. There was a suggestion of possible benefit on EDSS. This result must be interpreted most cautiously, particularly without evidence of benefit on other disability measures. In the 4.0–5.5 range, where improvement was seen, the EDSS score is determined primarily by distance a participant walks. A participant's motivation and degree of examiner encouragement can be affected by expectation effects, as can the cerebral and sensory system scores that also improved. The benefit on two MTR measures could be random false positives among the dozens of outcome variables examined.

Lack of inhibition of gadolinium enhancing lesions was surprising given the pleotropic anti-inflammatory actions of MSCs. One possibility is MSCs lack MS-relevant anti inflammatory activity in vivo despite suggestive data from in vitro and animal studies. Or, possibly, the cell dose was insufficient. There were few data to guide dose selection when this study was designed. The target dose, $2 \times 10^6$ MSCs/kg body weight, was based on a dose effective in treating graft-versus-host disease following allogeneic hematopoietic stem cell transplantation,[15] and was anticipated to be safe and reliably achievable based on our experience with MSC transplantation for several conditions.[16] Also, multiple administrations might be more effective.[17]

Third, there is no current consensus concerning optimal delivery route. We elected to administer cells IV because of ease of administration and data supporting ability of MSCs to traffic from blood to injured or inflamed central nervous system in animal models of stroke, brain tumor, traumatic brain injury, spinal cord demyelination, and MS.[1] This route has been employed in most studies of MSC transplantation in MS.[1] Although the lungs trap many cells following IV injection, numerous cells reach systemic circulation and become widely distributed in experimental models and human studies.[18] Intra-carotid delivery of MSCs, which has been explored in multiple system atrophy[19] but not in MS to date, raises microembolization concerns. Some studies have utilized an intrathecal, or combined IV and intrathecal routes.[2022] Finally, we utilized cryopreserved cells thawed just before infusion. Although viability of the infused cell product exceeded 95% in all cases, recent studies suggest that, immediately after thawing, cryopreserved MSCs have cytoskeletal changes,[23] impaired immunomodulatory function,[24,25] altered in vivo trafficking,[26] and increased T-cell mediated apoptosis.[23] Thus, harvesting cells directly from culture or allowing recovery time after thawing appears prudent.

Another cell production issue in designing the planned phase 2 study is use of FBS in our culture protocol. Although we received regulatory approval based on our previous experience, validation of a xenon-free culture protocol for future studies is underway. Preliminarily, MSCs from five normal donors have been culture-expanded in parallel in low glucose DMEM containing 10% FBS or 5% human platelet lysate (PLUS supplement). Mean yields after three passages were more than tripled and culture times nearly halved with platelet lysate, with equivalent purity.[27]

We utilized bone marrow-derived MSCs based on our previous experience, but important differences might exist between MSCs from different tissue sources, e.g. adipose tissue and bone marrow, a point not yet systematically investigated. A related issue is whether MSCs should be autologous or can/should be isolated from donors without MS. We used autologous cells to avoid potential concerns with transmitting infection or cancer from the donor. Also, although MSCs are relatively non-immunogenic permitting allogeneic administration,[15,28] some studies indicate repeated administration stimulates rejection[29] – an issue if multi-dosing is planned. Conversely, "universal donor" MSCs have the advantage of an "off the shelf" reagent with consistent properties, and would not be subject to putative deficient immunomodulatory or repair-promoting properties associated with MS. While some studies indicate that MSCs isolated from MS subjects and non-MS controls exhibit similar growth in culture, differentiation potential, surface antigen expression, and immunomodulatory properties,[30–32] others report notable functional differences,[33–35] suggesting administration of MSCs from a donor without MS might be preferable. This key issue is unresolved and is the subject of ongoing ancillary studies.

In summary, this phase 1 trial supports the feasibility, safety, and tolerability of autologous MSC transplantation in MS. Future trials to assess efficacy more definitively are warranted.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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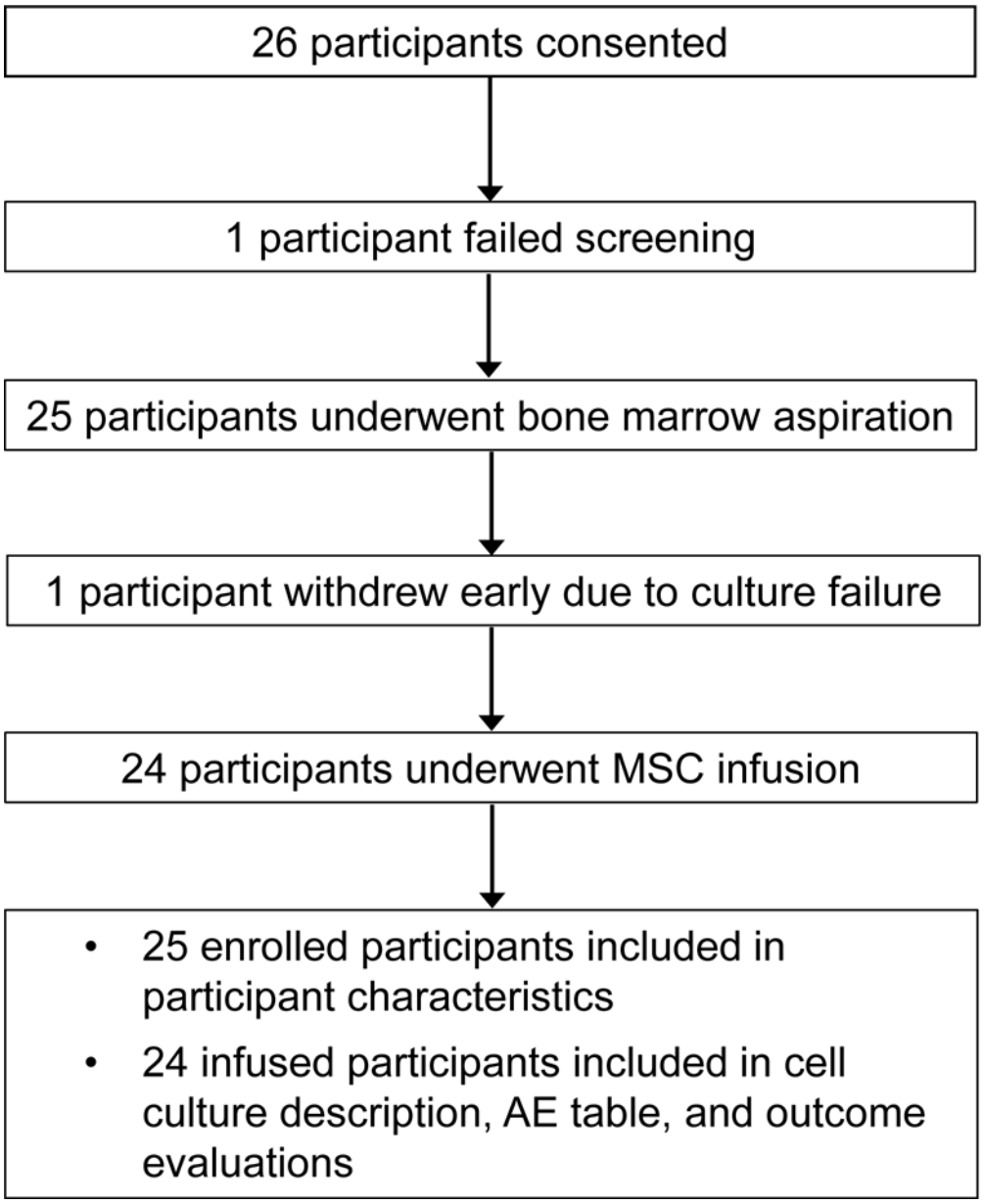


**Figure 1.**
CONSORT diagram.
MSC = mesenchymal stem cell, AE = adverse event

## Table 1

Characteristics of participants who underwent bone marrow aspiration and MSC culture (screening visit)

| Characteristic | Participants (n=2 5) |
|---|---|
| Age, years – mean (SD) | 46.4 (5.2) |
| Sex | 17 female / 8 male |
| Disease course | 11 RR / 14 SP |
| Disease duration, years – mean (SD) | 15.4 (9.0) |
| MS disease activity or worsening in the prior two years – n, (%) [a] | MS relapse 16 (64.0), EDSS or FSS worsening 11 (44.0), MSFC component worsening 17 (68.0), brain MRI lesion activity 10 (40.0), cervical spine lesion activity 1 (4.0) |
| EDSS – median (range) | 6.0 (3.0–6.5) |
| Current disease treatment – n, (%) | interferon-beta 6 (24.0), glatiramer acetate 7 (28.0), no treatment 12 (48.0) |
| MRI with gadolinium-enhancing lesions – n, (%) | 6 (25.0) |
| T2-hyperintense lesion volume, ml – mean (SD) | 21.5 (12.0) |
| Brain parenchymal fraction – mean (SD) | 0.777 (0.039) |

[a]The total exceeds 100% because some participants qualified based on more than one criterion.

EDSS = Expanded Disability Status Score, FSS = functional system score, MS = multiple sclerosis, RR = relapsing-remitting, SD = standard deviation, SP = secondary progressive

## Table 2

Cell culture kinetics and yield (n=24)

| Parameter | Mean ± SD (range) |
|---|---|
| Initial yield following Percoll centrifugation of bone marrow aspirate, $10^8$ cells | 5.05 ± 2.72 (1.52–11.27) |
| Final yield following cell-expansion, $10^8$ cells | 2.74 ± 1.29 (0.86–5.86) |
| Culture duration, days | 30.0 ± 11.3 (16–62) |
| Percent $CD105^+/CD73^+$ | 97.0 ± 2.1 (90.6–99.7) |
| Percent $CD45^+/CD14^+$ | 0.36 ± 0.46 (0.00–1.79) |
| Infused dose, $10^6$ cells/kg | 1.9 ± 0.2 (1.3–2.0) |
| Viability: trypan blue exclusion of administered cells | 98.6 ± 0.9 (95.2–99.5) |

There was one culture failure that was terminated after 28 days due to poor growth and atypical cell morphology.

## Table 3

Adverse events during or after autologous MSC infusion among participants who underwent infusion (n=24)

| Events | Number (%) of participants |
|---|---|
| Any event | 22 |
| Any severe event[a] | 3 (12.5)[c] |
| Any event leading to discontinuation of trial participation | 0 |
| Any serious event | 4 (16.7)[c] |
| Deaths | 0[d] |
| Most frequently reported events [b] | |
| Muscle spasticity | 11 (45.8) |
| Urinary tract infection | 10 (41.7) |
| Multiple sclerosis relapse | 7 (29.2) |
| Fall | 7 (29.2) |
| Multiple sclerosis | 5 (20.8) |
| Vision blurred | 5 (20.8) |
| Upper respiratory tract infection | 5 (20.8) |
| Balance disorder | 4 (16.7) |
| Nasopharyngitis | 3 (12.5) |
| Depression | 3 (12.5) |
| Diarrhoea | 3 (12.5) |
| Blood thyroid stimulating hormone decreased | 2 (8.3) |
| Back pain | 2 (8.3) |
| Fatigue | 2 (8.3) |
| Paraesthesia | 2 (8.3) |
| Anxiety | 2 (8.3) |
| Migraine | 2 (8.3) |
| Headache | 2 (8.3) |

[a] CTCAE grade ≥3

[b] reported by ≥2 participants

[c] Events classified as both severe and serious included: hospitalizations of two participants for treatment of a urinary tract infection with associated temporary worsening of neurologic symptoms, two hospitalizations of one participant for depression, hospitalization of one participant for management of hyperglycemia following IV methylprednisolone treatment of an MS relapse. None were considered related to mesenchymal stem cell transplantation.

[d] Two deaths occurred, 6 months and 40 months after completing the study, not considered related to mesenchymal stem cell transplantation.

**Table 4**

Exploratory efficacy results (n=24 for all values unless otherwise specified[a])

| | Screening | Month-1 | Baseline | Month 1 | Month 2 | Month 3 | Month 6 |
|---|---|---|---|---|---|---|---|
| **Participant self-reported outcome** | | | | | | | |
| Global well-being by visual analog scale, mm from the left | | | | | | | |
| Mean ± SD | 73.2 ± 17.2 | NA | 66.6 ± 15.9 | 67.9 ± 15.0 | 67.8 ± 16.0 | 58.6 ± 21.8 | 68.2 ± 17.0 |
| **Clinician-assessed outcomes** | | | | | | | |
| Expanded Disability Status Scale | | | | | | | |
| Mean ± SD | 5.2 ± 1.4 | 5.3 ± 1.4 | 5.3 ± 1.3 | 5.1 ± 1.5 | 4.9 ± 1.8 | 5.1 ± 1.7 | 5.0 ± 1.7 |
| Median (range) | 6.0 (3.0–6.5) | 6.0 (3.0–6.5) | 6.0 (3.0–6.5) | 6.0 (2.0–6.5) | 6.0 (1.5–6.5) | 6.0 (2.0–6.5) | 6.0 (2.0–6.5) |
| Multiple Sclerosis Functional Composite-4[b] | | | | | | | |
| Mean ± SD | −0.17 ± 0.60 | −0.13 ± 0.62 | −0.14 ± 0.61 | −0.09 ± 0.57 | −0.07 ± 0.57 | −0.12 ± 0.56 | −0.13 ± 0.58 |
| Timed 25-foot walk, sec | | | | | | | |
| Mean ± SD | 9.8 ± 4.9 | 10.2 ± 5.5 | 10.1 ± 5.0 | 9.8 ± 4.9 | 10.2 ± 5.0 | 11.6 ± 6.8 | 11.4 ± 6.9 |
| 9-hole peg test, sec | | | | | | | |
| Right, mean ± SD | 28.4 ± 11.6 | 28.5 ± 12.0 | 30.3 ± 21.1 | 29.7 ± 15.9 | 28.8 ± 17.4 | 28.1 ± 14.7 | 27.8 ± 14.1 |
| Left, mean ± SD | 27.8 ± 9.7 | 28.8 ± 11.7 | 28.3 ± 9.6 | 27.9 ± 9.7 | 27.6 ± 8.8 | 26.9 ± 8.7 | 27.9 ± 9.7 |
| Paced auditory serial addition test, n correct | | | | | | | |
| Mean ± SD | 45.3 ± 11.4 | 49.0 ± 10.0 | 47.9 ± 10.2 | 49.5 ± 10.3 | 49.8 ± 9.2 | 51.1 ± 8.6 | 50.8 ± 8.8 |
| Sloan low contrast letter acuity, binocular, n correct | | | | | | | |
| 100%, mean ± SD | 56.6 ± 4.6 | 56.3 ± 4.1 | 56.4 ± 5.2 | 56.7 ± 4.6 | 55.9 ± 5.5 | 55.7 ± 5.7 | 56.3 ± 5.2 |
| 2.5%, mean ± SD | 32.5 ± 9.6 | 31.3 ± 8.3 | 31.3 ± 9.7 | 31.1 ± 9.2 | 29.0 ± 9.2 | 30.7 ± 8.7 | 30.8 ± 9.4 |
| 1.25%, mean ± SD | 17.0 ± 12.5 | 15.5 ± 11.6 | 16.5 ± 12.1 | 16.6 ± 11.4 | 16.5 ± 9.8 | 14.0 ± 9.0 | 13.9 ± 9.4 |
| **MRI outcomes** | | | | | | | |
| Gadolinium enhancing lesions, n | | | | | | | |
| Mean ± SD | 0.58 ± 1.53 | 0.63 ± 1.20 | 0.75 ± 0.90 | 0.96 ± 1.57 | 0.92 ± 1.89 | 0.67 ± 1.37 | 0.79 ± 1.41 |
| Median (range) | 0 (0–7) | 0 (0–5) | 0.5 (0–3) | 0 (0–6) | 0 (0–6) | 0 (0–5) | 0 (0–5) |
| Proportion of scans with gadolinium-enhancing lesions | | | | | | | |
| n (%) | 6 (25) | 8 (33) | 12 (50) | 9 (38) | 8 (33) | 7 (29) | 8 (33) |

| | Screening | Month-1 | Baseline | Month 1 | Month 2 | Month 3 | Month 6 |
|---|---|---|---|---|---|---|---|
| Number of new or enlarged T2-hyperintense lesions (compared to the Screening MRI), n | | | | | | | |
| Mean ± SD | NA | 0.63 ± 0.92 | 0.92 ± 1.25 | 1.29 ± 1.78 | 1.38 ± 1.81 | 1.54 ± 2.23 | 1.79 ± 2.48 |
| Median (range) | NA | 0 (0–3) | 0.5 (0–3) | 0 (0–6) | 0 (0–6) | 0 (0–8) | 0 (1–10) |
| T2-hyperintense lesion volume, ml | | | | | | | |
| Mean ± SD | 21.6 ± 12.2 | 21.8 ± 12.5 | 22.2 ± 13.0 | 22.5 ± 13.0 | 22.1 ± 13.0 | 22.2 ± 13.0 | 22.0 ± 12.8 |
| T1-hypointense lesion volume, ml | | | | | | | |
| Mean ± SD | 3.44 ± 3.20 | 3.36 ± 3.11 | 3.41 ± 3.13 | 3.36 ± 3.10 | 3.32 ± 3.00 | 3.32 ± 3.02 | 3.31 ± 2.98 |
| Normalized brain volume (brain parenchymal fraction) | | | | | | | |
| Mean ± SD | 0.776 ± 0.039 | 0.775 ± 0.040 | 0.775 ± 0.039 | 0.774 ± 0.039 | 0.773 ± 0.040 | 0.774 ± 0.040 | 0.772 ± 0.041 |
| Gray matter fraction | | | | | | | |
| Mean ± SD | 0.460 ± 0.020 | 0.460 ± 0.020 | 0.460 ± 0.020 | 0.459 ± 0.020 | 0.459 ± 0.021 | 0.457 ± 0.021 | 0.458 ± 0.021 |
| Whole brain mean diffusivity, $\times 10^{-6}$ $mm^2/S$ | | | | | | | |
| Mean ± SD | 836.7 ± 33.8 | 841.1 ± 33.0[a] | 840.9 ± 29.7 | 845.1 ± 32.6[a] | 844.3 ± 33.3 | 843.9 ± 35.3[a] | 845.4 ± 33.4 |
| Whole brain fractional anisotropy | | | | | | | |
| Mean ± SD | 282.8 ± 11.3 | 282.5 ± 10.4[a] | 281.5 ± 10.4 | 281.8 ± 10.6[a] | 279.9 ± 11.7 | 282.2 ± 11.9[a] | 282.0 ± 10.5 |
| Whole brain magnetization transfer ratio | | | | | | | |
| Mean ± SD | 37.51 ± 2.56 | 37.49 ± 2.60 | 37.44 ± 2.66 | 37.50 ± 2.61 | 37.52 ± 2.62 | 37.62 ± 2.58 | 37.64 ± 2.59 |
| **Visual evoked potentials** | | | | | | | |
| P100 latency, average of both eyes, msec | | | | | | | |
| Mean ± SD | 133.3 ± 17.3 | 135.5 ± 19.1 | 133.1 ± 19.8 | 135.0 ± 19.2 | 135.3 ± 18.3 | 135.9 ± 18.5 | 137.1 ± 18.8 |
| **Optical coherence tomography** | | | | | | | |
| Retinal nerve fiber layer thickness, average of both eyes, microns | | | | | | | |
| Mean ± SD | 72.7 ± 7.6 | 72.8 ± 7.6 | 72.9 ± 8.2 | 72.6 ± 7.8 | 72.0 ± 7.7 | 72.3 ± 7.8 | 72.2 ± 7.7 |
| Macular volume, average of both eyes, $micron^3$ | | | | | | | |
| Mean ± SD | 9.38 ± 0.46 | 9.36 ± 0.47 | 9.34 ± 0.50 | 9.34 ± 0.47 | 9.35 ± 0.44 | 9.35 ± 0.45 | 9.31 ± 0.51 |
| Foveal thickness, average of both eyes, microns | | | | | | | |
| Mean ± SD | 261.78 ± 12.86 | 261.20 ± 12.58 | 260.68 ± 13.95 | 260.50 ± 12.96 | 260.89 ± 12.26 | 261.01 ± 12.50 | 259.72 ± 14.14 |

SD = standard deviation, NA = not available, because not measured at that visit

[a] n=23

[b] Timed 25-foot walk, 9-hole peg test, paced auditory serial addition test, low-contrast letter acuity