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Cell therapy with placenta-derived mesenchymal stem cells for secondary progressive multiple sclerosis patients in a phase 1 clinical trial

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Mesenchymal stem cell (MSC) has attracted significant attention in clinical research due to their immunomodulatory properties and potential to reduce inflammation in autoimmune disorders, such as multiple sclerosis (MS). This study evaluates the safety and feasibility of placenta-derived MSCs (PLMSCs) in five participants with secondary-progressive multiple sclerosis (SPMS). The primary outcomes focused on safety and tolerability, assessed through adverse event monitoring over six months. Secondary exploratory outcomes included clinical, imaging, and immunological measures. Patients underwent baseline evaluations and follow-up assessments comprising cognitive and psychological assessments, expanded disability status scale (EDSS), clinical signs, diffusion tensor imaging (DTI), functional MRI (fMRI), cytokine levels (IL-10, IL-6, IL-17, TNFα), and CD20/CD19 B cell marker analysis. No serious complications were noted, except for temporary headache in two patients, which was resolved with tablet. Results demonstrated sustained improvements in clinical outcomes, as indicated by significant reductions in EDSS scores ($P < 0.0001$), cognitive and psychological assessments, and radial diffusivity (RD) indices ($P = 0.0186$) in DTI metrics over six months. Furthermore, fMRI analysis showed significant enhancements in brain connectivity and cognitive function. Immunologically, CD20/CD19 B cell markers decreased significantly ($P = 0.0077$), and anti-inflammatory cytokine IL-10 increased alongside reductions in pro-inflammatory TNFα, IL-6, and IL-17 ($P < 0.0001$) three months post-therapy. These findings suggest PLMSC transplantation is safe and feasible in SPMS patients. While exploratory outcomes indicate potential clinical and immunological benefits, this phase 1 trial was not designed to assess efficacy. Larger, controlled phase II trials are warranted to validate these preliminary observations and investigate PLMSCs' therapeutic potential in MS.

Keywords Multiple sclerosis, Placenta-derived mesenchymal stem cells, Cell therapy, Brain mapping, Diffusion tensor imaging, Cognitive and psychological evaluations

Abbreviations

3D	Three-dimensional

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AD	Axial diffusivity
BM-MSCs	Bone marrow-derived MSCs
BVMT-R	Brief visuospatial memory test rev
CNS	Central nervous system
COWAT	Controlled oral word association task
CP	Cerebral palsy
CST	Cortico-Spinal tract
CVLT-II	California verbal learning test-II
D-KEFS	Delis-Kaplan executive function system
DMEM-LG	Dulbecco's modified eagle medium–low glucose
DMN	Default mode network
DTI	Diffusion tensor imaging
EDSS	Expanded disability status scale
FA	Fractional anisotropy
FBS	Fetal bovine serum
fMRI	Functional MRI
GMPs	Good manufacturing practices
BM-MSCs	Bone marrow derived MSCs
HBV	Hepatitis B virus
HCV	Hepatitis C virus
H&E	Hematoxylin & Eosin staining
HIV	Human immunodeficiency virus
HTLV	Human T-lymphotropic virus
ISCT	International society for cell therapy
IV	Intravenous
LPC	Lateral parietal cortex
MACFIMS	Minimal assessment of cognitive functions in MS
MSCs	Mesenchymal stem cells
MSC-Evs	MSC-derived extracellular vesicles
MSC-NP	MSC-derived neural progenitor
MD	Mean diffusivity
MNCs	Mononuclear cells
MS	Multiple sclerosis
Mpfc	Medial prefrontal cortex
OR	Optic radiation
PCC	Posterior cingulate cortex
PD-1	Programmed death-1
PLMSCs	Placenta-derived MSCs
PPMS	Primary progressive MS
PMS	Progressive MS
RD	Radial diffusivity
RRMS	Relapsing–remitting MS
RTX	Rituximab
SCs	Stem cells
SPMS	Secondary-progressive MS
TIP	Topology-informed pruning
UC-MSCs	Umbilical cord-derived MSCs
WB	Whole brain
FSS	Fatigue severity scale
SCL-90-R	Symptom checklist-90-Rev
SDMT	Symbol- digit modalities test
PASAT	Paced auditory serial addition test
PCC	Posterior cingulate cortex
JLO	Judgment of line orientation test

With 2.3 million people affected globally, MS is the most frequent immune-mediated and inflammatory disease of the CNS, which causes progressive neurodegeneration and demyelination of axons[1].

Current research and therapeutic strategies increasingly emphasize a more holistic approach, aiming to balance the interplay between remyelination and inflammation in MS, promoting regeneration while maintaining optimal levels of inflammation[2]. Mesenchymal stem cells (MSCs), including fetal-derived MSCs, have shown potential in addressing these needs by exerting anti-inflammatory, immunomodulatory, and neuroprotective effects, as well as enhancing remyelination[3,4].

In particular, placenta-derived MSCs (PLMSCs) express a higher amount of CD106 marker that increases MSCs immune modulation, along with expressing programmed death ligand 1/2 (PD-L1/2), which mediates T cell cycle suppression and proliferation. PLMSCs can be easily and non-invasively isolated from discarded pregnancy tissues, are abundant, have no ethical issues, and can differentiate into various neural cell lineages[5,6]. Additionally, studies have shown that PLMSCs exhibit more potent immunosuppressive behavior than adult MSCs[7–9]. In addition, more immunomodulatory functions of PLMSCs than other fetal-derived MSCs have been documented in in-vitro studies[10,11].

Despite these promising findings, the impact of MSCs on various aspects of MS remains unclear. Therefore, we designed a phase I clinical trial to assess the safety and tolerability of intravenous injection of PLMSCs in SPMS patients who show no response to conventional remedies.

To assess the effects of the PLMSC transplantation, we planned to use various techniques, such as DTI, for tracking white matter integrity, fMRI to study brain connectivity patterns, as well as cognitive & psychological evaluations (MACFIMS, FSS, and SL-90), and blood tests for cytokine and B cell marker evaluations.

In DTI metrics, the diffusion properties of water molecules in the brain are measured[12], consisting of fractional anisotropy (FA),and axial diffusivity(AD), RD, and mean diffusivity (MD)[13,14]. DTI indices provide greater specificity in identifying demyelination and axonal injury compared to conventionally used MRI techniques[15].

fMRI allows researchers to study how the brain's connectivity and activation patterns change before and after treatment, providing insights into mechanisms of neural regeneration and repair that may underlie cognitive or behavioral changes. Studying functional connectivity provides insights into neuroplasticity and recovery processes, especially in neurological disorders like MS, where disruptions in connectivity are evident[16].

Previous studies have shown that stem cell therapy in ischemic stroke patients enhances cortical activation on fMRI, suggesting that the therapy may induce plastic changes at the synaptic and neuronal levels[17,18].

As far as we know, the effects of PLMSC transplantation on various outcomes in SPMS patients before and after injection have not been evaluated in previous studies. Our hypothesis is that the clinical, cognitive, psychological, imaging, and immunological outcomes could be improved with PLMSCs in comparison to baseline.

Materials and methods

Study designs

The present open-label phase I research was carried out in the MS Clinic of Sina and Shariati Hospital, (Tehran province). The trial was registered with ClinicalTrials.gov (NCT06360861) and the Iranian Registry of Clinical Trials (IRCT20210614051576N1). The first trial registration occurred on 25/07/2021.

MS patients were diagnosed and managed according to "McDonald's criteria[19] and Iran's diagnostic and treatment protocols". Those with admittance to the MS Clinic of Sina Hospital, were deemed to have the eligibility for cell therapy based on the inclusion and exclusion criteria outlined in Table 1. Five SPMS patients underwent cell therapy with PLMSCs.

Informed written consent was acquired from all patients prior to the administration of MSCs.

Patients were recruited from July 25, 2021, to July 2, 2022, as follows.

SPMS patients were initially screened and underwent baseline phases and physical and neurological analyses. Counting blood factors (e.g., hemoglobin, platelets, and white blood cells), biochemical tests (ALT and AST), partial thromboplastin time, and partial thromboplastin time were the screening tests.

Moreover, concurrent medications or pre-treatment with steroids were not used in patients three months before the injection and until the end of the clinical study; only standard treatment with rituximab (RTX) was administered, as it was ethically prohibited to discontinue medication in patients. RTX has a half-life of approximately 18–20 days in patients with normal renal function; however, its biological effects, including B cell depletion, persist for 6–9 months due to the prolonged elimination of CD20 + B cell and delayed reconstitution of the B cell population[20–23].

The patient's selection process was based on the inclusion and exclusion criteria, as well as biochemical and blood tests to ensure the absence of bacterial and viral diseases, among others. Before transplantation, the patients' demographic characteristics, including age, sex, duration of disease, current treatment, annual attack, and EDSS score rate were obtained from their clinical documents in the MS Research Center as shown in Table S1-S3.

Patients were evaluated by a specialist physician for their health and the absence of concurrent diseases (such as viral or bacterial infections) through blood and biochemical tests before cell injection and one, three, and six months afterward. Following this, cytokine analysis was performed before cell injection and at one and three months later.

Inclusion criteria	Exclusion criteria
Age of 17–45 years	Pregnancy or breastfeeding
SPMS patients with no response to their DMT and reluctance to intensify for more potent treatments. (i.e., despite medication, their disease continued to progress)	Positive results for patients from screening tests of hepatitis C /B, human immunodeficiency virus (HIV), and human T cells lymphotropic virus (HTLV)
Currently taking Rituximab	Previous use of cytotoxic substances by patients during 3 months before the trial
Diseases lasting over 2 and below 16 years	Coagulation disorders, severe anemia (hemoglobin < 8 mg/dl), prior cell infusion, renal insufficiency, and a history of malignancy
	Existence of severe and irretrievable disease existing with a probable life expectancy of < 6 months
	Presence of accompanying liver disorders, e.g., liver failure or elevated liver enzymes
	Presence of rheumatoid arthritis, ulcerative colitis, or Crohn's disease
	Comorbid psychiatric conditions or other diseases causing cognitive impairment, such as dementia

Table 1. Study inclusion and exclusion criteria.

The six-month follow-up was chosen based on the timing of subsequent RTX administration. Patients received the RTX drug at the end of the clinical trial.

Prior to MSCs injection of MSCs, Cognitive and psychological evaluations were performed by a clinical psychologist for patients. DTI and fMRI were the other procedures.

Using an IV cannula, a single dose of PLMSCs was injected into the patients.

This research project (including placenta donation and PLMSCs manufacturing process) received approval from the "Ethical Committee of Tehran University of Medical Sciences" for Education, Culture, and Research (IR.TUMS.MEDICINE.REC.1400.197) and (IR.TUMS.NI.REC.1400.048). Figure 1 illustrates the study design and patient flow within the clinical trial.

Isolation and expansion of PLMSCs

Placenta specimens were obtained antiseptically from normally full-termed gestations during cesarean section parturition. The donors presented written informed acquiescence forms agreeing with the national ethical protocols for research on SCs and regenerative medicine. After reviewing the donor's medical histories, namely laboratory tests and physical examinations, blood was sampled for complementary laboratory tests. Applicable laboratory tests were applied to evaluate the presence of HBV, HCV, HIV, CMV, HTLV, EBV, Toxoplasma, and venereal diseases[24].

The manufacture of GMP-grade PLMSCs was based on a previously described procedure by the authors[25]. After aseptic double-bagging of the placenta in the operative suite, it was conveyed in a cool box to the Research Institute for Oncology, Hematology& Cell Therapy Facility, Shariati Hospital. In brief, the placenta was devoid of the umbilical cord (UC) and exterior membranes.

The cut tissue samples were fully rinsed with normal saline and minced into fine pieces approximately ~ 1 cm wide × 0.5 cm deep with scissors. Pieces of the tissues underwent digestion with collagenase type I in HBSS for 1–2 h at 37 °C. The digestion was stopped by adding DMEM-low glucose (Biowest Company, USA) containing FBS (Biowest Company, USA). After that, mononuclear cells were separated by centrifuging at 350 g for 5 min. After washing and resuspending of the separated MNCs in culture media with 10% FBS, they were counted, seeded into culture flasks, and cultured in a CO_2 incubator. After 48 h, unattached cells were expelled and each flask was repleted with fresh culture media. Two times a week, the media were changed and subcultures of PLMSCs were prepared at 80–90% confluency with trypsin–EDTA (Thermo Fisher Scientific, USA). Upon the confluence of the Passage 2 (P2) or P3 cells, most of the cells were cryopreserved for subsequent uses, and only two T75 flasks were reseeded to be propagated and characterized further. The proliferated cells are usable for mesodermal differentiation analyses and cell characterization with flow cytometry.

For the characterization of PLMSCs, CD marker expression (CD34, CD73, CD105, and CD90) was examined through flow cytometry (Shariati laboratory, Iran).

Their multilineage differentiation potential of PLMSCs was assessed using osteogenesis, adipogenesis, and chondrogenesis differentiation media, following the guidelines of the International Society for Cell Therapy (ISCT). Briefly, 6 × 10^4 cells were cultured in induction media for 14 days, after which osteogenesis (bone-like matrix), adipogenesis (lipid vacuoles), or chondrogenesis were characterized by using Alizarin Red, Oil Red O, and Hematoxylin–Eosin staining, respectively.

No changes occurred in the MSC identity markers and MSC capability in expansion and differentiation, as in our tests of pilot examinations. Microbial infection, Gram staining, mycoplasma, and endotoxins were the tests applied to the final product.

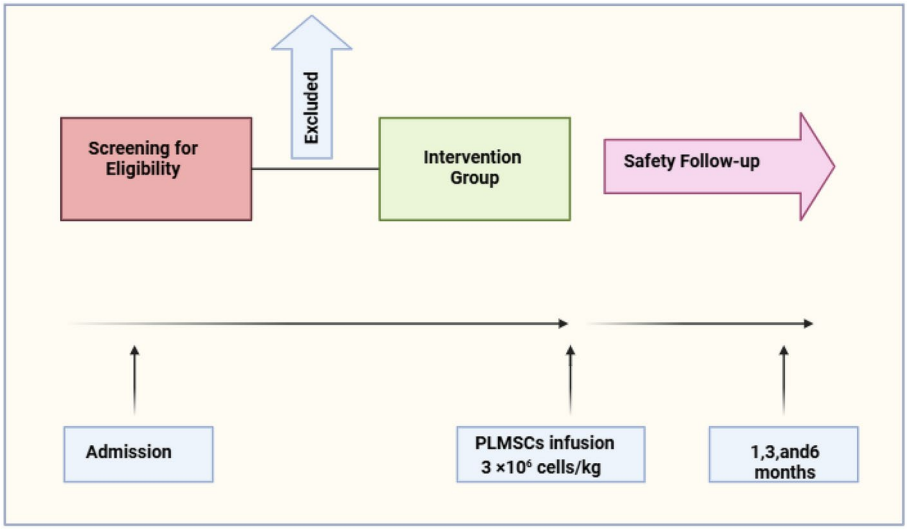

Fig. 1. Study design and patient follow-up in the clinical trial. Created with BioRender.com.

The stable status of chromosomes was ensured by analyzing the third subcultures of PLMSCs with G-band karyotyping. The final step was the resuspension of cells in the 2% human albumin plus 1U/ml heparin-supplemented normal saline.

Measurements of outcomes

Primary outcomes

In this phase I clinical trial, the primary outcomes were to evaluate the safety and tolerability of intravenous PLMSCs transplantation in SPMS patients, with no response to the conventionally used remedies. Each patient received 3×10^6 cells/kg through IV injection (cephalic and basilic veins). The slow injection of suspended cells (for 15 min) was followed by continually monitoring of participants' vital signs, such as blood pressure, temperature, and heart rate. To prevent complications like allergic reactions, 100 mg of hydrocortisone was given before the injection. To determine the safety of cell injection, early side effects such as fatigue, headache, skin rash, chills, nausea, blood pressure changes, myocardial infarction, respiratory issues, fever during injection, and anaphylactic shock were monitored continuously for 24 h. Additionally, safety and tolerability were assessed continuously throughout the entire follow-up period.

Secondary outcomes

Secondary outcomes focused on improvements in clinical symptoms, cognitive & psychological problems as well as a decrease in systemic inflammation and the CD20/CD19 markers of the B cells. All patients were monitored through clinical visits and their clinical status was assessed using the EDSS by a neurologist, with measurements recorded at 0-, 1-, 3-, and 6-months post- injection.

Additionally, DTI, fMRI, and cognitive and psychological evaluations were conducted at months 0, 3, and 6.

Cytokine levels (TNFα, IL-17, IL-10, and IL-6) and changes in B lymphocyte subpopulations (CD19+, CD20+) were monitored through flow cytometry at baseline (month 0) and then at 1- and 3-months post-injection.

Cognitive evaluations

The cognitive performance of participants was measured with the minimal assessment of cognitive functions in MS (MACFIMS) test battery in a Persian translation, the reliability of which was certified for Iranian MS patients in 2012[26].

The MACFIMS battery includes ten subtests designed to evaluate various aspects of cognitive function. The California Verbal Learning Test-II (CVLT-II), was utilized to assess Patients' verbal learning and memory deficits, while the Paced Auditory Serial Addition Test (PASAT) measured processing speed, cognitive flexibility, and calculation ability. The Symbol- Digit Modalities Test (SDMT) evaluated processing and motor speed, and the Judgment of Line Orientation Test (JLO) assessed visuospatial ability. Verbal fluency was measured with the Controlled Oral Word Association Task (COWAT), while executive functions were evaluated through the Delis-Kaplan Executive Function System (D-KEFS) Sorting and Descriptive Tests. Additionally, the Brief Visuospatial Memory Test Rev (BVMT-R) was employed to assess visuospatial learning. To further evaluate memory, delayed recall tests from both the BVMT-R and CVLT-II were administered[26].

Psychological evaluations

The validated Persian version of the Symptom Checklist-90-Rev (SCL-90-R) was employed for psychological assessments[27]. The SCL-90 collects nine subscales (with 90 items) to assess psychoticism, obsessive–compulsive behavior, paranoid ideation, phobic anxiety, hostility, anxiety, depression symptoms, interpersonal sensitivity, and somatization in the past week. Each item was scored on a 5-point Likert scale, where the scores ranging from 0 to 4. To calculate SCL-90 Global Severity, the summed score of all subscales was divided by 9.

Fatigue was examined by the Persian version of the Fatigue Severity Scale (FSS)[28], a 9-item scale assessing fatigue severity in the last 2 weeks. Individual items are scored from 1 to 7, with a total score from 9 to 63. More severe fatigue is indicated by greater FSS scores.

The cognitive and psychological functions of participants were evaluated at the base of the study (before intervention), 3 and 6 months after cell injection. All tests were run by the same expert psychologist to reduce the assessment bias.

MRI acquisition

The images of MR were prepared on a scanner (3Tesla SIEMENS Prisma). An imaging procedure was applied to the subjects consisting of sagittal 3D T2-weighted and fluid-attenuated inversion.

recovery sequence (FLAIR), three-dimensional (3D) T1-weighted, DTI, and T2-weighted at National Brain Mapping Lab, Tehran, Iran, with a 64-channel head coil. The entire brain was imaged as 3D T1-weighted with the imaging parameters of FOV (field of view) = 220mm, TR = 1840 ms, Voxel size of $0.9 \times 0.9 \times 0.9$ mm, TE = 3.55 ms, Flip angle = 7 degree, and Slice thickness = 0.9 mm with no gap. The DTI images were obtained with a 2D- EPI diffusion sequence utilizing these parameters: TE = 79 and TR = 6200 ms, and diffusion sampling (64 directions) was selected in total. The b-value, in-plane resolution, and slice thickness were 2000s/mm2, 1.04 mm, and 2 mm, respectively. Whole brain FLAIR parameters were TE = 130 ms, TI = 2338 ms, and TR = 8000 ms with a voxel size of $1.0 \times 1.0 \times 1.2$ mm, and FOV = 220.

Image processing

Tractography DTI images were processed and subjected to tractography with DSI Studio (http://dsi-studio.labsolver.org/). The DTI images underwent visual inspection for quality control and then corrected for eddy current distortion and motion. The b value table was examined automatically with a quality control system to assure its

precision, followed by calculating the diffusion tensor (Fig. S1). For all fiber tracts, the fiber was tracked automatically using the anatomy prior to a tractography atlas, followed by a deterministic fiber- tracking algorithms and randomly selecting the anisotropy. An angular threshold of 15–90 degrees was determined through random selection, with a step size of 0.3 mm. The direction of propagation with 80% of the prior direction was averaged to smooth the fiber trajectories.

Whole brain (WB) A seeding area was located at WB. Tracts with lengths of below 50 or above 600 mm were rejected, calculating 2000 tracts in total.

Cortico-spinal tract (CST) After placing a seeding area at CST, tracks with lengths below 50 or above 600 mm were rejected, calculating 500 tracts in total. False connections in the tractography were eliminated through topology-informed pruning (TIP) to the tractography over 16 iteration(s).

Optic radiation (OR) A seeding area was located at the OR. Tracks with lengths below 100 or above 600 mm were rejected, calculating 300 tracts in total. False connections in the tractography were eliminated through TIP to the tractography over 16 iteration(s).

Corpus callosum (CC) After placing a seeding area at CC, tracks with lengths below 50 or above 600 mm were rejected, calculating 700 tracts in total. False connections in the tractography were eliminated through TIP to the tractography over 16 iteration(s).

Volumetry The MRI image processing was conducted using the established protocols of BrainSuite software (Version 15b; http://brainsuite.org/). This software utilizes anatomical data from both surface models and brain image volumes to accurately align the subject's cerebral cortex with an atlas, resulting in the automatic extraction of surface models. The final parcellation determined the overall volumes of the entire brain, encompassing both white matter and gray matter, through the utilization of surface and volume registration (SVReg). Additionally, it included the measurement of subcortical gray matter regions, such as the amygdala and thalamus.

Lesion analysis
The 3D FLAIR image was segmented manually beside additional control on the T2 weighted image with MRICron (http://www.mccauslandcenter.sc.edu/mricro/mricron). This software provides tools for accurately defining and restricting areas of brain lesions. A neurologist who received training from experienced neuroradiologists specialized in MS performed each manual segmentation. The lesions were detected as areas of increased brightness compared to the surrounding tissue. They had a minimum size of three voxels and were found in the deep white matter, juxtacortical, and infratentorial regions. After the process of segmentation, the total number of lesions, their respective volumes, and the overall lesion load were computed.

FMRI acquisition

fMRI data were acquired using a 3 T MRI scanner (SIEMENS Magneto Prisma Fit Scanner), furnished with a head -neck coil(64-channel). The following are the acquisition parameters for the functional data, namely Repetition Time (TR)/Echo-Time (TE): 1030/30, Flip Angle: 80°, Matrix Size: $384 \times 384 \times 400$ mm^3, Voxel Size: $3 \times 3 \times 1$ mm^3. This configuration provided whole-brain coverage during acquisition. In addition, T1-weighted anatomical images were also acquired using a "3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence". The sequence had the following parameters: Voxel Sizes: $1 \times 1 \times 0.6$ mm^3, FOV: 256×256 mm^3, TR/TE: 2250/3.5 ms, Matrix: 256×256 mm^2, Number of Slices: 225 contiguous sagittal slices, and Flip Angle: 7°.

Preprocessing of fMRI data
The fMRI data underwent preprocessing using the CONN toolbox version [22a] in MATLAB. The analysis was applied to five cases, each of which had three fMRI sections: before treatment, 3, and 6 months after cell injection. The preprocessing steps included realignment, slice-timing correction (ST), spatial normalization into the standard MNI space, smoothing with FWHM Gaussian kernel of mm (=8), artifact detection, and bandpass filtering (0.01–0.1 Hz) focused on low-frequency fluctuations. Denoising procedures were also applied, which included physiological noise correction, motion-related artifact removal, and signal regression for white matter and cerebrospinal fluid.

Visualization of functional connections
BrainNet Viewer was employed to visualize three-dimensional functional connections and compare weight matrices.

Blood test

Patients were monitored for cytokines serum levels such as IL-6, TNFα, IL-10, and IL-17, and changes in B lymphocyte subpopulation counts (CD19 + , CD20 +) on months 0, 1, and 3.

Statistical analysis

Data analysis was done with the SPSS Software (IBM Group, version 26.0, USA) and Graph Pad Prism version 9.4.1. In this study, the normality and long normality tests were adopted to describe the normally distributed raw data.

Due to the presence of time, one-way repeated measures ANOVA was employed in this study to analyze data as mean ± S.D. Statistical differences were expressed as $P < 0.05$. The data of biomarkers levels and DTI analysis (FA, MD, AD, and RD) before and after treatment were evaluated by the Wilcoxon signed-rank test, and correlations were evaluated using linear regression analysis.

Data related to cognitive and psychological function were presented by median (interquartile range). The changes in each test score during the three time points of the study were examined by Friedman test, for which the Wilcoxon signed rank test was employed as the post-hoc test.

In addition, the resulting functional connectivity matrices were statistically analyzed using NBS-predict. This approach allowed examining any change in functional connectivity after some time and assessing the impact of our treatment protocol. Using the NBS routine, NBS-predict identifies linked constituents in the sub-threshold edge collection. The machine learning algorithm uses connection values as features existing at the sub-threshold edge in the main connection constituent. The following are the major parameters: repeated CV = 50, seed = 42, K-fold = 10, p-value = 0.01, hyper-parameter optimization steps = 5, permutation = 5000, and grid search. Besides, potential confounding factors were controlled by regressing age and gender. Extra examinations were done by choosing the edges with a P-value < 0.01. For disconnected subnetworks, the brain networks were assessed utilizing a threshold value of 1.

Results

Clinical results

In this research, qualified subjects were identified by the initial screening of 12 participants, assigning 5 cases to the research. In this study, no severe complications associated with PLMSCs were observed. After the injection, an insignificant headache was observed in only two patients (n # 1 and n # 5), which was mild and temporary and resolved with an acetaminophen tablet after 1 h. A medical doctor, who was uninformed on resolving all indications, recorded these initial incidents, and no reports of other adverse events were seriously received during the 6-month follow-up period and one year after. EDSS decreased in the first month after cell injection and then showed a declining trend in two patients in the third month while remaining constant in the rest of the patients (Fig. 2). Table S1 describes EDSS changes for three years before the injection and up to one year after MSCs injection ($P < 0.0001$).

The doctor did not share the obtained data with the researchers till the research completion. Table S2 illustrates the history of DMTs for SPMS patients before and after MSC injection. The follow-up stage continued till January 22, 2023.

Results of cognitive and psychological evaluations

The changes in MACFIMS' subtests score at the base, 3, and 6 months after intervention are presented in Table S4 in the supplementary data file.

The results of BVMT-R (P: 0.05), DKEFS-sorting (P: 0.02), CVLT-II (P: 0.04), and DKEFS-descriptive (P: 0.02) revealed significant differences between the three-time points in this research. The post-hoc analysis for time points pairwise showed a significant increasing trend from base to the 6 months after intervention in the cases of PASAT (P: 0.04), BVMT-R (P: 0.04), DKEFS-sorting (P: 0.05), and DKEFS-descriptive (P: 0.04). Additionally, the changes of descriptive (P: 0.04) and sorting (P: 0.03) DKEFS from 3 to 6 months of study were increasing and significant, as shown in Fig. 3.

As reported in Table S5, the changes in hostility (P: 0.02) and fatigue (P: 0.02) scores were significant statistically between the three-time points of the study. More analysis for time points pairwise highlighted meaningful decreases in fatigue score from baseline to the third month (P: 0.04) and from the baseline to the

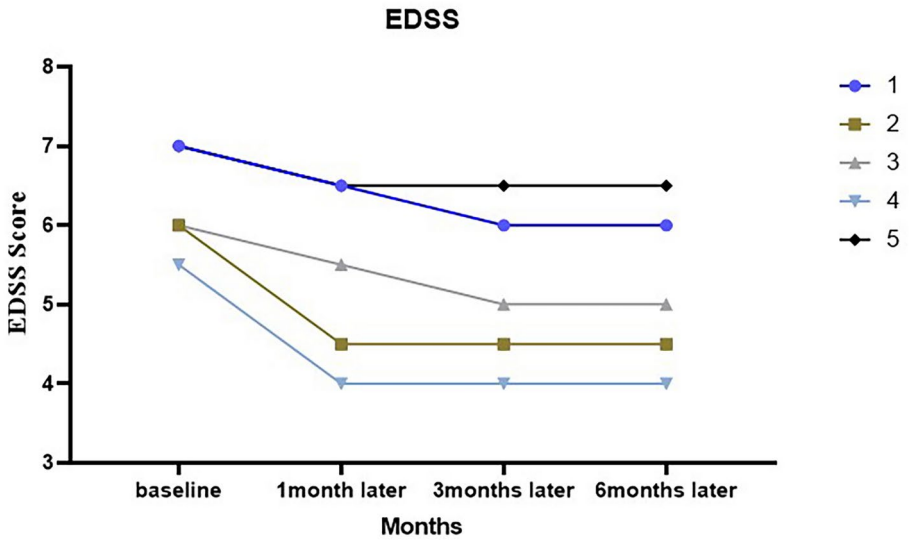

Fig. 2. EDSS scores of patients with SPMS on baseline and 1, 3, and 6 months after SC injection ($P < 0.0001$).

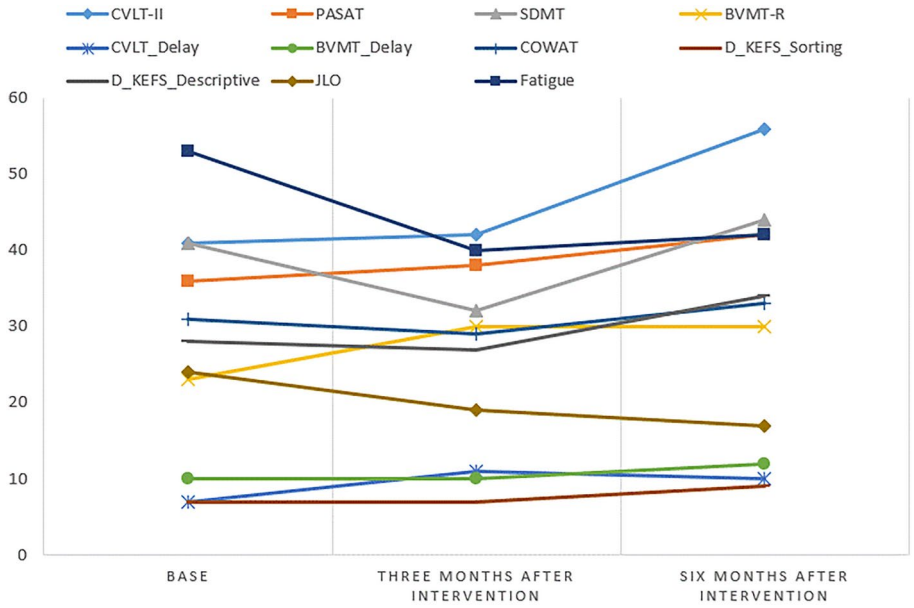

Fig. 3. The changes in cognitive performance and fatigue scores of participants at the base of the study and 3 and 6 months after the intervention.

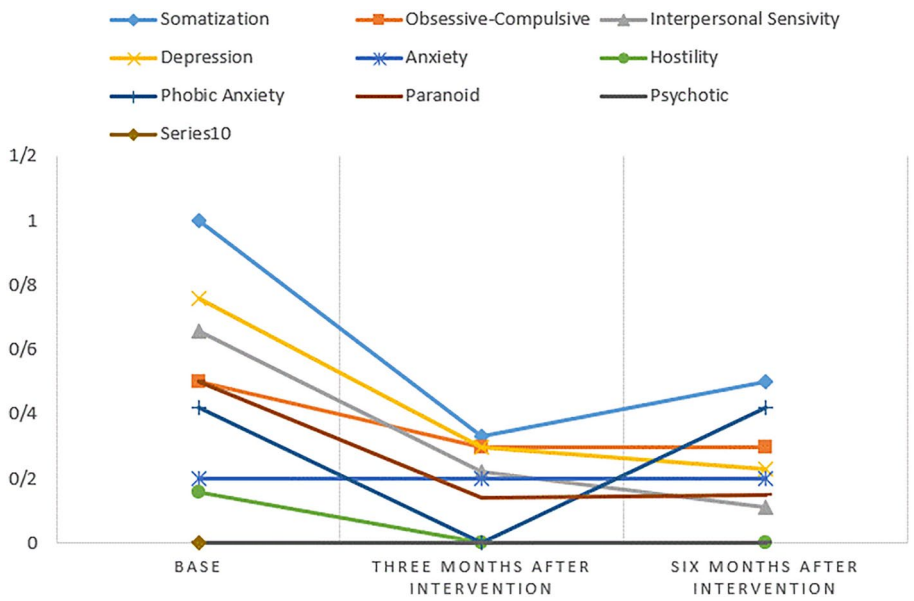

Fig. 4. The changes in psychological functions of participants at the base, 3, and 6 months after the intervention.

sixth month of the study (*P*: 0.04). On the other hand, somatization (*P*: 0.04) and paranoid (*P*: 0.04) scores had a significant decreasing trend from baseline to the sixth month of the study. No significant differences were found for the SCL-90 Global Severity scale ($P > 0.05$), as illustrated in Figs. 3 and 4.

Data are presented as the median of each of the MACFIMS subtests and fatigue scores. *P*-values were estimated using Friedman's test.

The trends of BVMT-R (*P*: 0.05), D-KEFS-Sorting (*P*: 0.02), CVLT-II (*P*: 0.42), and D-KEFS-Descriptive (*P*: 0.02), showed significant increases and significant, while fatigue exhibited a significant decreasing trend (*P*: 0.02).

Data are presented as the median of each of the psychological tests. *P*-values are calculated using Friedman's test. The trend for hostility showed significant decrease (*P*: 0.02). Additionally, somatization ($P = 0.04$) and paranoid ideation ($P = 0.04$) scores significantly decreased from baseline to 6 months.

MRI characteristics

Brain volume changes between baseline and follow-up

The volume of basal ganglia, NAWM, and abnormal-appearing white matter were not significantly different between 3 and 6 months and between baseline and 6 months. Furthermore, no notable decreases were observed in the overall brain size or WM volume. The reduction in cortical gray matter volume was also not statistically significant.

Changes in lesion characteristics between baseline and follow-up

T2 lesion count, volume, and burden in periventricular, Juxta-cortical, deep white matter, and infra-tentorial regions were not significantly different between the baseline and 6-month follow-up. Table S6 and S7 summarize trends in lesion formation (gadolinium enhancement and new T2 lesions) in MRI for patients three years before the injection and up to one year after MSCs injection.

Global alterations of DTI-metrics between baseline and over the follow-up

Tractography-based analysis No significant differences were seen in diffusivity indices over 6 months in CST, OR , and CC.

(Fig. S1 & 2) and Table S8.

ROI or region of interest analysis The ROI analysis of the NAWM at the left hemisphere showed a significant decrease in RD ($p = 0.0186$) between baseline and 6 months and between 3 and 6 months of follow-up. While MD, AD, and FA showed positive trends in most analyses, the changes were not statistically significant.

(Fig. S2, RD and Table S8, B).

Results of fMRI (comparing the whole brain network with NBS predictions)

For visualizing the subnetwork comprising relevant edges, applying the most stringent feature weight threshold (1.0) identified 113 brain areas connected by 143 increased connections in the MS group 6 months after treatment (Fig. 5). The nodal degree of connection between brain areas is illustrated in the supplemental data file. The left default mode network, the left Parietal, and the right Para hippocampal were detected as the areas with the utmost magnitude (nodal degrees = 9 and 8). These regions are involved in key cognitive functions often impaired in MS, including memory, spatial processing, and default mode activity.

Visualization of the functionally increased connection subnetwork (weight a threshold of 1) in MS patients is shown on a 3D brain surface produced with the BrainNet Viewer**.** In addition, nodes and edges are respectively illustrated by size and color based on the nodal degree and their weight. The increased connection is mainly localized in the temporal, frontal, and visual regions, as well as in the subcortex. A greater degree of connection is observed in both hemispheres.

Results of laboratory tests

Flow cytometry analysis of patient samples collected prior to and after PLMSC therapy revealed a decrease in CD20/CD19 markers within the B lymphocyte population (Fig. 6 and with further information available in the supplementary data file, Fig. S3A and S3B). This suggests a reduction in B cells following the administration of cell and standard treatment.

The levels of cytokines were assessed prior to and following cell therapy at the beginning (month 0) and at 1- and 3-months post-treatment, as depicted in Fig. 7 and Table S9. The results demonstrated that level of the anti-inflammatory cytokine (IL-10) considerably rose 3 months after cell therapy, while level of pro-inflammatory cytokines (IL-17, IL-6, and TNFα) decreased significantly. One patient exhibited an increase in IL-6 levels, most likely as a result of a COVID-19 infection.

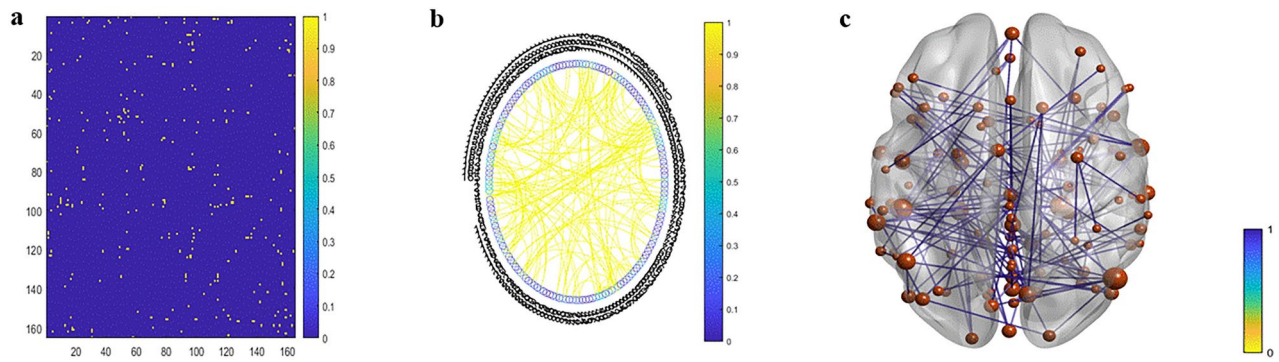

Fig. 5. (**a**) The weighted adjacency matrix and (**b**) circular network show increased connections in the MS group 6 months after treatment. The colored edges in both figures and the nodes in the circular network reflect their weights and nodal degrees (weight threshold = 1). (**c**) The changes in functional connectivity of participants 6 months after treatment.

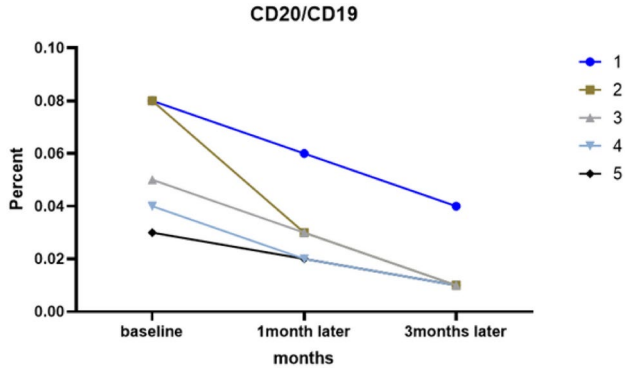

Fig. 6. Measured dual expression levels of CD20/CD19 B cells in patients with SPMS on baseline and after 1 & 3 months of SC injection ($P = 0.0077$).

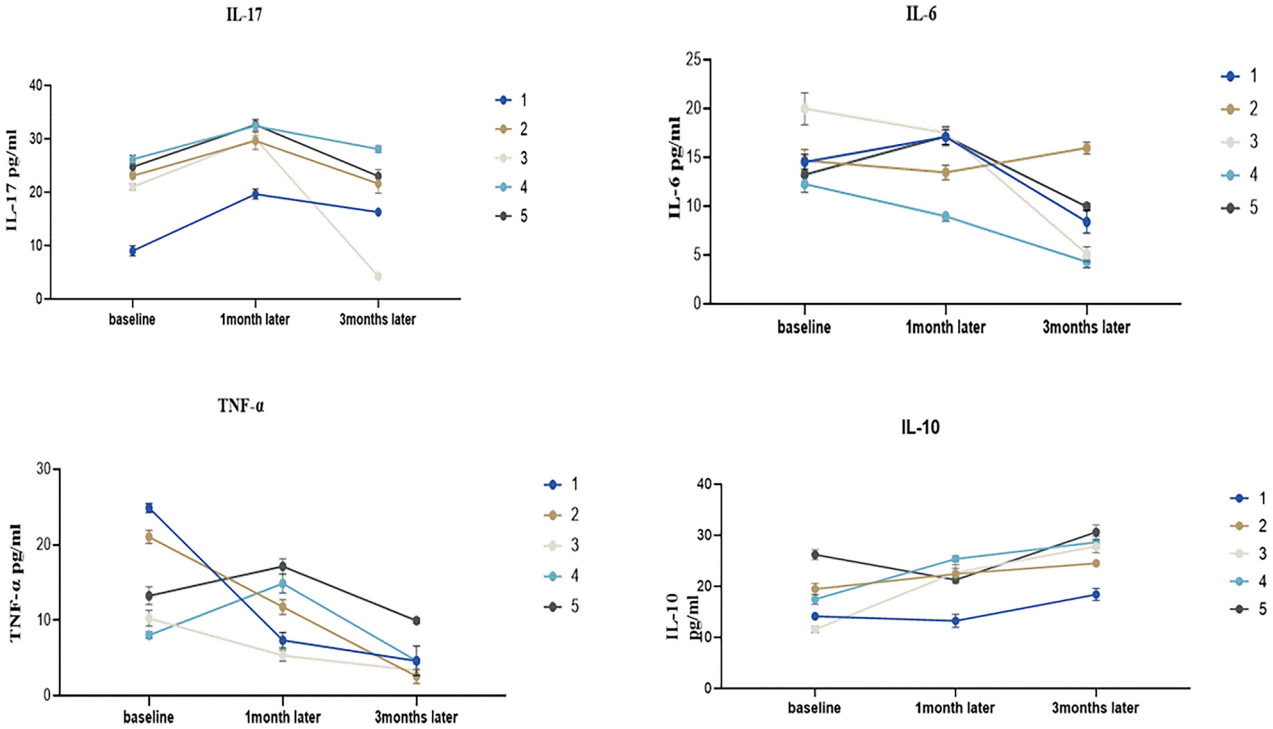

Fig. 7. Changes in serum concentrations of biomarkers (IL-6, TNF-α, IL-17, and IL-10) of the patients on 0 (baseline), 1, and 3 months following the cell infusion. The analyzed biomarkers prior to and 3 months after injecting SCs demonstrated significant reductions in TNF-α, IL-6, and IL-17 in most of the patients. IL-10 levels increased in all patients ($P < 0.0001$). The participants evidenced no disease activity throughout the 6-month treatment period.

Discussion

The current investigation evaluated the safety and tolerability of PLMSC injection in patients with SPMS. No reports of serious adverse events were received during the follow-up period and one year after.

The results of our research, consistent with previous studies, suggest potential improvements in patients' clinical conditions, including stable EDSS scores, reduced dysfunction, alleviated MS symptoms, and decreased systemic inflammation. This may suggest a potential reparative effect in SPMS patients[29,30]. However, these findings should be interpreted with caution, as this Phase I study was not powered to detect clinical effectiveness and primarily focused on safety and feasibility. Our study indicates some positive trends in cognitive and psychological status, as well as a reduction in RD in the left DTI-ROI analysis of NAWM. Additionally, we observed enhanced brain connectivity and a decline in the dual expression of CD20/CD19 B cells. However, we acknowledge that the limited cohort size warrants caution in drawing definitive conclusions, and further research is needed to better understand these findings in the context of MSC transplantation.

Several clinical trials confirm the safety and tolerability of MSC transplantation in MS patients[3,14,30]. For instance, Riordan et al. showed that seven doses of UC-MSCs improved EDSS scores, reduced dysfunction, and revealed inactive MRI lesions after one year[29]. Similarly, Lublin et al. administered placental MSCs (PDA-001) to 16 MS patients. During the one-year follow-up, lesion counts did not worsen, and EDSS scores remained stable or improved for most patients[30].

Furthermore, our results suggest that PLMSC injection may benefit MS patients, particularly as a new treatment for cognitive and psychological disorders in patients with SPMS who exhibit a high prevalence of these disabilities. Cognitive impairment, affecting 50–70% of SPMS patients, significantly impacts daily life and quality. Its prevalence varies due to research settings and disease progression[31]. Additionally, studies suggest that newly diagnosed SPMS patients face a higher risk of developing psychological issues[32,33]. A survey by Jones et al. of 4,178 MS patients found that those with SPMS had a significantly higher likelihood of experiencing depression compared to other forms of MS[34]. Currently, there is no definitive treatment for cognitive and psychological disorders in MS patients; thus, searching for effective interventions is particularly valuable. Based on the authors' information, the effects of PLMSC injection on the cognitive and psychological functions in SPMS patients have not been previously investigated. However, limited studies have reported improvements in memory or dementia following PLMSC injection in rats[35,36].

DTI analysis for the NAWM revealed reduced RD in the left hemisphere between baseline and follow-up. Notably, Post-transplantation changes in DTI parameters within lesional areas may indicate the resolution of inflammation and edema, as well as various degrees of repair mechanisms, affecting RD and AD values[37].In a study by Amanat et al., UC-MSC treatment significantly improved WM integrity in cerebral palsy patients over one year[2]. Fernandez et al. found potential clinical and radiological benefits from autologous adipose-derived MSCs in 19 MS patients after 12 months[38]. In addition, Yamout et al. suggested potential clinical efficacy and safety of BM-MSC injections in 10 MS patients, but no radiological improvements were noted after one year[39].

In our study, fMRI revealed increased connectivity between memory, spatial processing, and default mode networks, which correlated with improved cognition and memory based on neuropsychological evaluations. These findings suggest that PLMSCs infusion may facilitate neural repair and regeneration in MS patients, leading to measurable cognitive improvements. This is consistent with emerging evidence supporting the role of MSCs in promoting neurogenesis and synaptic plasticity[35,40,41]. Our study contributes to the expanding body of literature on SC therapy's neurological outcomes in MS, highlighting the necessity for further research in larger cohorts to establish its effects and long-term efficacy. Furthermore, the default mode network (DMN) is a group of brain areas that show preferential activity upon no goal-directed activity and are deactivated when sensorimotor or cognitive tasks are competed. The medial prefrontal cortex (mPFC) hippocampus, posterior cingulate cortex (PCC), right and left or lateral parietal cortex (LPC), and precuneus are among the DMN regions that exhibit synchronous fMRI activity patterns. The DMN is thought to support ongoing, or default, brain functions like self-referential mental activity and autobiographical memory retrieval because these brain regions are associated with "internally focused tasks." A number of structural and functional studies have revealed abnormal DMN activity in MS patients[42,43].In research by Petrou et al., autologous MSCs were used in 48 progressive MS (PMS) patients. The study found that MSCs-intrathecal (IT) transplantation reduced relapse rates, improved monthly T2 lesion load on MRI. Positive outcomes were also observed in cognitive tests, fMRI, and optical coherence tomography scans, with a significant increase in motor network activity noted in the MSCs-IT group after one year[44].

Research indicates that the administered MSCs may exert their effects through a combination of systemic immunomodulation and potentially direct actions at the sites of damage[45,46].

Preclinical and clinical studies suggest that MSCs' survival in vivo may be limited, and their positive effects could be due to their role in regulating tissue homeostasis and inflammation through the secretion of various paracrine factors[3,47].

In our trial, the IL-10 levels increased, while TNFα, IL-6, and IL-17 levels decreased in most patients after three months, supporting the anti-inflammatory role of MSCs[48]. Further studies are needed to explore these mechanisms.

Furthermore, elevated IL-17 levels have been found in brain lesions and blood of MS patients, correlating with active disease[49,50]. Therefore, reducing IL-17 may help alleviate inflammation and promote repair in MS[49]. Jung et al. found that MSC-derived extracellular vesicles (MSC-EVs) can convert Th17 cells into low-IL-17 producers, improving symptoms and reducing Th17 cells in an MS mouse model[51]. This study suggests a correlation between elevated IL-17 levels and increased cognitive dysfunction and physical disability in MS patients, highlighting the role of pro-inflammatory cytokines in worsening these disabilities[52].

In the present study, we observed a decline in CD20/CD19 markers in B lymphocytes following cell therapy, indicating a reduction in the B cell population. This is significant because B cell antigen presentation is crucial in the pathogenesis of MS. There is increasing interest in therapies targeting B cells[53,54], particularly MSCs, which inhibit B cell activation and promote regulatory B cells (Bregs)[55,56]. However, RTX was administered one month before cell infusion, making it unethical to discontinue the drug. Its pharmacokinetics suggest that the effects on B cell depletion are long-lasting, with reconstitution typically taking over 6 months[20–23]. Thus, the observed reduction in CD20/CD19 B cell markers is likely attributable to RTX's sustained biological activity rather than a transient effect.

In this study, we opted for IV administration, as it offers several advantages: it is safer, less invasive than intrathecal injection, well-tolerated, allows for systemic release anti-fibrotic of and anti-inflammatory factors, and permits the administration of repeated MSCs doses over a short course of cell therapy[2,48].

In summary, our results suggest possible neuroprotective effects from PLMSC administration. We acknowledge that the limited cohort size, short follow-up, and lack of control groups necessitate caution in interpreting these findings. Larger studies with repeated cell injections, longer follow-up periods, control groups, and objective

biomarkers are needed to thoroughly assess neuro-inflammation and neuronal regeneration following MSC transplantation.

Conclusions and future insights

The present trial suggests potential clinical effectiveness and possible indications of neuroprotection in the short-term following the injection of PLMSCs in SPMS patients. However, further studies with larger cohorts are needed to validate these findings.

Our viewpoint is that a critical point is the timed SC injection in MS patients. As a new remedy in patients with MS, the effectiveness of PLMSCs can be improved through several high-dose injections in the initial stage of inflammation.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding authors on reasonable request.

Received: 12 October 2024; Accepted: 29 April 2025
Published online: 08 May 2025

References

1. Frahm, N., Hecker, M. & Zettl, U. K. Multi-drug use among patients with multiple sclerosis: A cross-sectional study of associations to clinicodemographic factors. *Sci. Rep.* **9**, 3743 (2019).
2. Amanat, M. et al. Clinical and imaging outcomes after intrathecal injection of umbilical cord tissue mesenchymal stem cells in cerebral palsy: A randomized double-blind sham-controlled clinical trial. *Stem Cell Res. Ther.* **12**, 439 (2021).
3. Shokati, A. et al. A focus on allogeneic mesenchymal stromal cells as a versatile therapeutic tool for treating multiple sclerosis. *Stem Cell Res. Ther.* **12**, 400 (2021).
4. Ebrahimi-Barough, S. et al. Standard operating procedure for the good manufacturing practice-compliant production of human endometrial stem cells for multiple sclerosis. *Methods Mol. Biol.* **2286**, 199–212 (2021).
5. Torre, P.d.l., Pérez-Lorenzo, M. & Flores, A.I. Human Placenta-Derived Mesenchymal Stromal Cells: A Review from Basic Research to Clinical Applications. *Stromal Cells - Structure, Function, and Therapeutic Implications* (2018).
6. George, S., Hamblin, M. R. & Abrahamse, H. Differentiation of mesenchymal stem cells to neuroglia: In the context of cell signalling. *Stem Cell Rev. Rep.* **15**, 814–826 (2019).
7. Yang, Z. X. et al. CD106 identifies a subpopulation of mesenchymal stem cells with unique immunomodulatory properties. *PLoS ONE* **8**, e59354 (2013).
8. Wu, M. et al. Comparison of the biological characteristics of mesenchymal stem cells derived from the human placenta and umbilical cord. *Sci. Rep.* **8**, 5014 (2018).
9. Wang, G. et al. Expression and biological function of programmed death ligands in human placenta mesenchymal stem cells. *Cell Biol. Int.* **37**, 137–148 (2013).
10. Abumaree, M., Abomaray, F., Alshabibi, M., AlAskar, A. & Kalionis, B. Immunomodulatory properties of human placental mesenchymal stem/stromal cells. *Placenta* **59**, 87–95 (2017).
11. Siddesh, S. E. et al. Placenta-derived mesenchymal stem cells (P-MSCs) for COVID-19 pneumonia-a regenerative dogma. *Stem Cell Investig.* **8**, 3 (2021).
12. Meoded, A., Poretti, A., Mori, S. & Zhang, J. Diffusion Tensor Imaging (DTI)☆, in *Reference Module in Neuroscience and Biobehavioral Psychology* (Elsevier, 2017).
13. Ontaneda, D. et al. Measuring brain tissue integrity during 4 years using diffusion tensor imaging. *AJNR Am. J. Neuroradiol.* **38**, 31–38 (2017).
14. Feng, J. et al. Exploratory MRI measures after intravenous autologous culture-expanded mesenchymal stem cell transplantation in multiple sclerosis. *Mult. Scler. J. Exp. Transl. Clin.* **5**, 2055217319856035 (2019).
15. Amiri, M. et al. Changes in diffusion tensor imaging indices in basal ganglia and thalamus of patients with Relapsing-Remitting Multiple Sclerosis and relation with clinical conditions: A case-control study. *Eur. J. Radiol. Open* **10**, 100465 (2023).
16. Jackson, G. D., Badawy, R. & Gotman, J. Chapter 23 - Functional magnetic resonance imaging: focus localization. In *Handbook of Clinical Neurology* (eds Stefan, H. & Theodore, W. H.) 369–385 (Elsevier, 2012).
17. Lee, J. et al. efficacy of intravenous mesenchymal stem cells for motor recovery after ischemic stroke: A neuroimaging study. *Stroke* **53**(1), 20–28. https://doi.org/10.1161/STROKEAHA.121.034505 (2022).
18. Ramos-Cabrer, P., Justicia, C., Wiedermann, D. & Hoehn, M. Stem cell mediation of functional recovery after stroke in the rat. *PLoS ONE* **5**, e12779 (2010).
19. Thompson, A. J. et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173 (2018).
20. Chisari, C. G. et al. Rituximab for the treatment of multiple sclerosis: A review. *J. Neurol.* **269**, 159–183 (2022).
21. Ng, C. M., Bruno, R., Combs, D. & Davies, B. Population pharmacokinetics of rituximab (anti-CD20 monoclonal antibody) in rheumatoid arthritis patients during a phase II clinical trial. *J. Clin. Pharmacol.* **45**, 792–801 (2005).
22. Leandro, M. J., Cambridge, G., Ehrenstein, M. R. & Edwards, J. C. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthrit. Rheum.* **54**, 613–620 (2006).
23. Welte, T. et al. Identification of covariates modulating B-Cell repopulation kinetics in subjects receiving rituximab treatment. *Arthrit. Rheumatol.* **75**, 2045–2053 (2023).
24. Aghayan, H. R. et al. GMP-Compliant Production of Human Placenta-Derived Mesenchymal Stem Cells. In *Stem Cells and Good Manufacturing Practices: Methods, Protocols, and Regulations* (ed. Turksen, K.) 213–225 (Springer, 2021). https://doi.org/10.1007/7651_2020_282.
25. Shokati, A. et al. Good manufacturing practices production of human placental derived mesenchymal stem cells for therapeutic applications: Focus on multiple sclerosis. *Mol. Biol. Rep.* **51**, 460 (2024).
26. Eshaghi, A. et al. Validity and reliability of a persian translation of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *Clin. Neuropsychol.* **26**, 975–984 (2012).
27. Akhavan Abiri, F. & Shairi, M. R. Validity and reliability of symptom checklist-90-revised (SCL-90-R) and brief symptom inventory-53 (BSI-53). *Clin. Psychol. Person.* **17**, 169–195 (2020).
28. Azimian, M., Farahani, A. S., Dadkhah, A., Fallahpour, M. & Karimlu, M. Fatigue severity scale: The psychometric properties of the persian-version in patients with multiple sclerosis. *Res. J. Biol. Sci.* **4**, 974–977 (2009).
29. Riordan, N. H. et al. Clinical feasibility of umbilical cord tissue-derived mesenchymal stem cells in the treatment of multiple sclerosis. *J. Transl. Med.* **16**, 1–12 (2018).

30. Lublin, F. D. 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.* **3**, 696–704 (2014).
31. Benedict, R. H. B., Amato, M. P., DeLuca, J. & Geurts, J. J. G. Cognitive impairment in multiple sclerosis: Clinical management, MRI, and therapeutic avenues. *Lancet. Neurol.* **19**, 860–871 (2020).
32. Bogosian, A., Morgan, M. & Moss-Morris, R. Multiple challenges for people after transitioning to secondary progressive multiple sclerosis: A qualitative study. *BMJ Open* **9**, e026421 (2019).
33. Bogosian, A. et al. Key demographics and psychological skills associated with adjustment to progressive Multiple Sclerosis early in the diagnosis. *Front. Rehabil. Sci.* **3**, 966133 (2022).
34. Jones, K. H. et al. A large-scale study of anxiety and depression in people with Multiple Sclerosis: A survey via the web portal of the UK MS Register. *PLoS ONE* **7**, e41910 (2012).
35. Cho, J. S. et al. Effect of placenta-derived mesenchymal stem cells in a dementia rat model via microglial mediation: A comparison between stem cell transplant methods. *Yonsei Med. J.* **59**, 406–415 (2018).
36. Yun, H. M. et al. Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1-42-infused mouse model of Alzheimer's disease. *Cell Death Dis.* **4**, e958 (2013).
37. Feng, J. et al. Exploratory MRI measures after intravenous autologous culture-expanded mesenchymal stem cell transplantation in multiple sclerosis. *Multiple Scler. J. Exper. Trans. Clin.* **5**, 2055217319856035 (2019).
38. Fernández, O. 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* **13**, e0195891 (2018).
39. Yamout, B. et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: A pilot study. *J. Neuroimmunol.* **227**, 185–189 (2010).
40. Zhou, W. et al. Exosomes derived from human placental mesenchymal stem cells enhanced the recovery of spinal cord injury by activating endogenous neurogenesis. *Stem. Cell Res. Ther.* **12**, 174 (2021).
41. Cao, N. et al. Clinical-grade human umbilical cord-derived mesenchymal stem cells reverse cognitive aging via improving synaptic plasticity and endogenous neurogenesis. *Cell Death Dis.* **8**, e2996–e2996 (2017).
42. Rocca, M. A. et al. Network damage predicts clinical worsening in multiple sclerosis: A 6.4-Year Study. *Neurol. Neuroimmunol. Neuroinflamm.* **8**, e1005 (2021).
43. Suzuki, J. et al. Bilateral cortical hyperactivity detected by fMRI associates with improved motor function following intravenous infusion of mesenchymal stem cells in a rat stroke model. *Brain Res.* **1497**, 15–22 (2013).
44. Petrou, P. et al. Beneficial effects of autologous mesenchymal stem cell transplantation in active progressive multiple sclerosis. *Brain* **143**, 3574–3588 (2020).
45. Mazzini, L. et al. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. *Exp. Neurol.* **223**, 229–237 (2010).
46. Uccelli, A., Moretta, L. & Pistoia, V. Mesenchymal stem cells in health and disease. *Nat. Rev. Immunol.* **8**, 726–736 (2008).
47. Shandil, R. K., Dhup, S. & Narayanan, S. evaluation of the therapeutic potential of mesenchymal stem cells (MSCs) in preclinical models of autoimmune diseases. *Stem. Cells Int.* **2022**, 6379161 (2022).
48. Hashemian, S. R. et al. Mesenchymal stem cells derived from perinatal tissues for treatment of critically ill COVID-19-induced ARDS patients: A case series. *Stem. Cell Res. Ther.* **12**, 91 (2021).
49. Kolbinger, F., Huppertz, C., Mir, A. & Padova, F. D. IL-17A and multiple sclerosis: Signaling pathways, producing cells and target cells in the central nervous system. *Curr. Drug. Targets* **17**, 1882–1893 (2016).
50. Tzartos, J. S. et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *Am. J. Pathol.* **172**, 146–155 (2008).
51. Jung, S. et al. Mesenchymal stem cell-derived extracellular vesicles subvert Th17 cells by destabilizing RORγt through posttranslational modification. *Exp. Mol. Med.* **55**, 665–679 (2023).
52. Rezaeimanesh, N. et al. The correlation between serum level of interleukin-17A with cognitive impairment and disability in multiple sclerosis patients. *Mult. Scler. Relat. Disord.* **80**, 105199 (2023).
53. Almatrafi, Y. M. et al. Efficacy and safety of rituximab in patients with multiple sclerosis: An observational study at a tertiary center in Makkah Saudi Arabia. *Neurosciences (Riyadh)* **27**, 65–70 (2022).
54. Fan, L. et al. Interaction between mesenchymal stem cells and B-cells. *Int. J. Molecular Sci.* **17**(5), 650. https://doi.org/10.3390/ijms17050650 (2016).
55. Yoshioka, S. et al. CCAAT/enhancer-binding protein β expressed by bone marrow mesenchymal stromal cells regulates early B-cell lymphopoiesis. *Stem. Cells* **32**, 730–740 (2014).
56. Xue, Q. et al. The immunomodulatory function of human amniotic fluid stromal cells on B lymphocytes. *J. Neurorestoratol.* **6**, 122–133 (2018).

Acknowledgements

The authors are grateful to all patients for their participation in this study. We sincerely appreciate Multiple Sclerosis Research Center and Research Institute for Oncology, Hematology& Cell Therapy Facility, Shariati Hospital for their help with providing facilities for this study. The authors thank the members of Shariati and Sina Hospital for their contributions to the study.

Author contributions

A.S, AN. M, J. A, and M. N contributed to the study conception and design. Material preparation, data collection and analysis were performed by A. S, M A.S, R.S, E.A, N.R, B.C, Z.G, and SA. M. M A.S, and S A. M were responsible for the reference selection. The first draft of the manuscript was written by A.S and A N. M, J. A, and M. N commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

Multiple Sclerosis Research Center supported this project with grant number 1400-1-148-52617 and Research Institute for Oncology, Hematology& Cell Therapy Facility, Shariati Hospital with grant number 1400-1-233-51589.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This study was conducted according to national ethical standards and laws and have been endorsed by the Ethics Committee at Tehran university of Medical Sciences. (Ref # IR.TUMS.HORCSCT.REC.1400.007).

Consent for publication

Not applicable.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-00590-6.

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