Safety and Effectiveness of Cell Therapy in Neurodegenerative Diseases: Take-Home Messages From a Pilot Feasibility Phase I Study of Progressive Supranuclear Palsy

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*Mesenchymal stromal cells (MSCs) are multipotent cells with anti-inflammatory properties. Here we tested the safety of MSCs in patients with progressive supranuclear palsy (PSP; ClinicalTrials.gov: NCT01824121; Eudracit No. 2011-004051-39). Seven patients were treated. To improve the safety, protocol adjustments were made during the performance of the study. The objectives of our work were: (1) to assess the safety of MSCs and (2) to identify critical issues in cell therapies for neurodegenerative diseases. Autologous MSCs from the bone marrow of PSP patients were administered through the internal carotid arteries. 1-year survival and number of severe adverse events were considered as safety endpoints. Clinical rating scales, neuropsychological assessments, gait and posture analysis, single-photon emission computed tomography, positron emission tomography, and brain magnetic resonance (BMR) were performed at different follow-up times. Peripheral blood levels of inflammatory cytokines were measured before and after cell infusion. Six of the seven treated patients were living 1 year after cell infusion. Asymptomatic spotty lesions were observed at BMR after 24 h in six of the seven treated patients. The last patient in the preliminary cohort (Case 5) exhibited transiently symptomatic BMR ischemic alterations. No severe adverse events were recorded in the last two treated patients. Interleukin-8 serum concentrations...
decreased in three patients (Case 2, 3, and 4). An adaptive study design, appropriate and up-to-date efficacy measures, adequate sample size estimation, and, possibly, the use of a cellular and/or allogeneic cell sources may help in performing phase II trials in the field.

**Keywords:** progressive supranuclear palsy (PSP), Parkinson's disease, cell therapy, mesenchymal stromal cells (MSCs), posture

## INTRODUCTION

Progressive supranuclear palsy (PSP) is a sporadic neurodegenerative disease, characterized by ocular motor dysfunction, postural instability, akinnesia, cognitive dysfunction, dysarthria, and dysphagia (Steele et al., 1964). The major pathological finding is the accumulation of tau protein in multiple brain regions, including the substantia nigra, globus pallidus, subthalamic nucleus, pontine tegmentum, and cerebellar dentate nucleus (Dickson et al., 2011). Neuroinflammation is an important factor in PSP progression (Bevan-Jones et al., 2019; Inci et al., 2020; Malpetti et al., 2021). Therefore, immunomodulatory disease-modifying therapies may be extremely useful in controlling PSP.

Newly developed, cell-based, advanced therapy medicinal products (ATMPs) have shown encouraging results in different clinical contexts. In particular, mesenchymal stromal cells (MSCs) are currently being used as immunomodulatory drugs in several phase III clinical trials on graft-versus-host disease (GvHD) after bone marrow (BM) transplantation (Gay et al., 2021). MSCs have been granted conditional market approval in Canada and New Zealand for the treatment of children with GvHD refractory to steroids (Cyranoski, 2012).

In 2010, we began preclinical and validation tests to develop treatments for severe and rare neurodegenerative diseases (Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico Ethics Committee approval 1464/10). In 2012, an autologous cell therapy product made of MSCs from the BM of PSP patients was developed following good manufacturing practices (GMP). We performed a phase I/II study [ISS Authorization Prot. N. 37519(11)-PRE 21-1111] to assess the safety and efficacy of MSCs in reducing neuroinflammation and rescuing neuronal survival and function.

In 2016, our group published results from a preliminary phase I study of five PSP patients who were treated with autologous BM MSCs. In the original experimental design, a blind randomized controlled crossover trial would have followed this preliminary phase. In the randomized trial, 20 patients would have been randomly assigned to receive MSC administration followed by a sham intervention or to receive a sham intervention followed by MSC administration, with a 6-month delay between the treatments. Following the preliminary phase I study, we substantially modified the protocol for cell infusion for safety reasons because intra-arterial MSC administration was associated with an intrinsic risk of microembolization. In our experience, this risk was invariably present in all treated patients, and one patient was symptomatic with transient ischemic brain magnetic resonance (BMR) lesions. To test the effectiveness of these modifications on the treatment safety profile, the Italian National Competent Authority requested that we treat three additional patients in the open, non-randomized phase before moving on to the subsequent phase.

In our study, we administered MSCs by infusion in the carotid arteries to exploit the ability of the cells to pass the blood–brain barrier and to exert their effects directly on brain tissues (Giordano et al., 2014). This form of administration would prevent the MSCs from being entrapped in the lung, as expected if systemic administration was used (Eversart et al., 2012; Sensebe and Fleury-Cappellesso, 2013; De Becker and Riet, 2016). Selective catheterization of the cerebral arteries was previously performed to deliver MSCs and hematopoietic progenitor cells to treat multisystemic atrophy (Lee et al., 2012) and Parkinson's disease (Brazzini et al., 2010), respectively. The most frequent adverse event recorded in the MSC clinical trial by Lee et al. (2012) was the presence of small ischemic lesions (<1 cm) on diffusion-weighted brain magnetic resonance imaging (MRI). These lesions were associated with the catheterization procedure.

The aims of the present study were to add further data to our previous work (Canesi et al., 2016) in order to assess the safety of intra-arterial administration of autologous MSCs in PSP patients and to identify critical issues and possible solutions for effective cell therapies in treating neurodegenerative diseases. We measured the survival at 1 year from cell administration and the number of severe adverse events as safety endpoints. To examine the effects of the treatment, we used clinical rating scales, neuropsychological assessments, and gait and posture analysis. We measured cytokine profiles in the peripheral blood, but due to logistic and ethical reasons and in consideration of the frailty of the patients, we could not perform liquor collection to assess the effect of MSC administration on cerebral inflammation. In addition, single-photon emission computed tomography (SPECT), positron emission tomography (PET), and BMR were performed before and after treatment. The overall goal of this study was to provide data to guide further phase II studies in the field.

## METHOD

### Protocol Approval and Patient Screening

This project complied with the mission of our hospital, which is dedicated to regenerative medicine and rare diseases. The protocol was authorized by the local Ethics Committee of Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico (Italy) and the National Competent Authority for phase I cell therapy at the National Health Institute (Istituto Superiore
di Sanità). The trial protocol was approved by the Italian Medicines Agency (Agenzia Italiana del Farmaco, AIFA). After the first preliminary phase, subsequent protocol amendments were authorized by the Italian National Competent Authority (AIFA) and by the local Ethics Committees. All patients gave their written informed consent. The trial is registered at ClinicalTrials.gov (NCT01824121). A detailed description of the study design, inclusion and exclusion criteria, BM collection, MSC isolation, and administration, as well as clinical (motor and neuropsychological) and neuroimaging assessments have been previously reported (Giordano et al., 2014; Canesi et al., 2016). The major changes in the protocol conduction were the number of patients to be included (three more in the preliminary phase I study) and the method for cell infusion (see the specific paragraph below).

Initially and periodically (at least once a month), the list of PSP patients was pre-screened by two researchers to select eligible patients based on the information in the most recently updated institutional clinical records. The candidate patients were contacted, and a screening visit was performed to assess the inclusion/exclusion criteria. Information was given to the patient, and informed consent was obtained. The informed consent form was signed by each patient before prior to the commencement of any study-related procedures.

Cell Manufacturing
Each patient underwent BM harvesting in an outpatient setting. MSC GMP production was performed at the Cell Factory of the public hospital Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico in Milan, Italy. The facility has maintained its manufacturing authorization (AIFA) in compliance with European GMP regulations to produce ATMPs since 2007 without interruption. The facility is a fully controlled plant for ATMP manufacturing; its characteristics as well as the main quality assurance procedures were previously described (Montemurro et al., 2015). Briefly, all manufacturing procedures were performed in a class A environment (Class II Type A2 Biological Safety Cabinet) with a class B surrounding environment. Microbial contamination was monitored using settle plates and volumetric active air sampling, and surfaces and operators were sampled with contact plates. In-continuous airborne particle monitoring was performed in the class A environment and during critical steps in the class B environment using automatic particle counters.

The procedures for manufacturing were developed by the authors. Briefly, unprocessed BM was directly seeded in alpha Modified Eagle Medium supplemented with 10% fetal bovine serum at a concentration of 50,000 total nucleated cells/cm² in a Cell Stack Chamber system (Corning, Lowell, United States). After 72 h, non-adherent cells were removed by washing with phosphate-buffered saline (Macopharma, Mouvaux, France) and completely changing the medium. Medium changes were also performed twice a week. On day 14 (±3), MSCs at P0 were detached using 25 mL/layer of trypsin (TrypLE Select, Gibco-Life Technologies, Carlsbad, CA, United States), transferred in bags, and washed by centrifugation (400 g, 12 min, no brake) in normal saline solution with 10% (vol:vol) human serum albumin (Kedrion, Castelvecchio Pascoli, Lucca, Italy).

Therefore, the supernatant was discarded and the cells re-seeded in cell stacks in the same culture conditions at a concentration of 4,000 MSCs/cm². The culture was stopped at 28 ± 3 days (passage 2), and the cells were detached and washed as at P0 and re-suspended in a solution containing normal saline solution with 10% (vol:vol) human serum albumin (Kedrion) and 10% (vol:vol) dimethyl sulfoxide (Bioniche Lifesciences, Inc., Belleville, ON, Canada). The cell product was cryopreserved at a concentration of 1–5 × 10⁶ cells/mL using a controlled-rate freezer (Nicool Plus, Air Liquide) programmed to freeze at −1°C/min and stored in bags (CryoMACS Miltenyi, Teterow, Germany) in the vapor phase of liquid nitrogen.

The quality criteria for releasing the final cell product were: number of cells > 1.5 × 10⁶ cells/kg of body weight, purity (% CD90+/105+/45 – cells) > 80%, viability (% P.I. – cells) > 80%; sterility; bacterial endotoxin < 0.25 E.U./mL, absence of Mycoplasma; normal karyotype.

On the day of the infusion, the cells were thawed at 37°C and normal saline solution with 10% (vol:vol) human serum albumin (Kedrion) and 12% anticoagulant citrate dextrose solution, solution A (Fresenius Kabi, Bad Homburg, Germany) were added to the suspension of thawed cells without removing DMSO. The final concentration of DMSO was 1% vol:vol. A visual inspection of the thawed cell suspension was performed using an optic microscope (100x) to exclude cell aggregates, visible impurities, or any other abnormalities just before infusion.

Cell Infusion
Each patient underwent neuroleptoanalgesia and was monitored by an anesthetist. MSCs were administered by the intra-arterial route, as described previously (Canesi et al., 2016). Briefly, using the Seldinger technique, catheterization was carried out via the right common femoral artery (or the left one in the event of difficulty in achieving arterial access) using a 6F Ultimim EV (St Jude Medical, MN, United States) introducer and a 5F Hinck or Simmons (Terumo Europe NV, Leuven, Belgium) diagnostic catheter.

An angiographic study of the cervical and intracranial arteries was performed using a 0.035-inch diameter, 150-cm long hydrophilic guide (Terumo Europe NV, Leuven, Belgium). Intravenous administration of heparin sodium (3,000–5,000 IU according to body mass) was completed. Subsequently, using a 260 cm exchange wire Easykit with a 0.35” or 0.38” diameter [Ab Medica S.P.A., Lainate (MI), Italy], a 90 cm 6F guiding catheter Envoy XB (Miami Lakes, FL, United States) was placed. The guiding catheter, flushed by heparinized saline, was positioned at the origin of both internal carotid arteries and at the origin of the widest vertebral artery. Once the guiding catheter was in place, a microcatheter Rebar 027 (130 or 145 cm) or Rebar 018 (153 cm; ev3/Covidiem, Irvine, CA, United States), steered by a 205 cm Transend EX 0.014 (Boston Scientific, Natick, MA, United States), was moved forward up into the internal carotid arteries. The microcatheter was placed just above the origin of the ophthalmic arteries and in the widest vertebral artery up to its V2 segment. The MSCs were then injected into the various locations (the left and
right internal carotid arteries and the widest vertebral artery) through the microcatheter using a pump at 70 ± 30 mL/h. The catheter was periodically flushed with heparinized normal saline solution.

Clinical and Neuropsychological Assessment

Patients were observed for the occurrence of adverse events during each protocol procedure and at all follow-up points.

After arterial catheterization, the patient was strictly monitored with the face arm speech test immediately after the procedure and then 3, 6, 18, and 24 h later with the aim for rapidly recognizing early signs of cerebral ischemia. BMR was performed 24 h after the procedure. If asymptomatic ischemic lesions were observed, anti-platelet therapy was continued and BMR was repeated after 14 days. In the case that no adverse event was reported, the patient was discharged 48 h after cell administration.

The following scales were used to assess treatment efficacy on neurological functions: the Unified Parkinson’s Disease Rating Scale (UPDRS) part III, motor score (Goetz et al., 2008), Hoehn and Yahr (H&Y) staging (Goetz et al., 2004), the PSP rating scale (PSP-RS; Golbe and Ohman-Strickland, 2007), and the Mini-Mental State Evaluation (Folstein et al., 1975). The scales were administered at baseline and at each follow-up point (1, 3, 6, and 12 months after cell administration). The clinical condition was classified as stable when the UPDRS and PSP-RS scores did not diminish by more than 30% compared to baseline and the H&Y staging did not increase by more than one point at the defined follow-up point.

Neuroimaging and Biomechanical Evaluation

All patients underwent longitudinal neuroimaging assessments, using brain MRI, striatal dopamine transporter SPECT, and PET. SPECT was performed with [1-123] ioflupane, a selective...
radioligand for dopamine reuptake transporters, and PET was performed with 2-fluoro-2-deossi-D-glucosio, as previously described (Canesi et al., 2016).

The biomechanical evaluation was completed at baseline and at the 6-month follow-up. The kinematic assessment of posture was performed as described in Palmisano et al. (2020). The assessment accounted for the patient's base of support and anthropometric measurements. For statistical analysis, the individual patients' measures were located at the tails of the distribution of the group of healthy subjects (below the 10th and above the 90th percentile). Healthy controls were chosen to have the same demographic and anthropometric characteristics of the patients, which have a large impact on kinematic assessment, as previously demonstrated (Palmisano et al., 2020).

### Inflammatory Cytokine Measurements in the Peripheral Blood of Progressive Supranuclear Palsy Patients

Peripheral blood (sera) levels of interleukin (IL)-6, IL-8, tumor necrosis factor-α, IL-1β, IL-5, IL-10, and interferon-γ were measured before and 72 h after MSC administration. Measurements were made using a high-sensitivity planar enzyme-linked immunosorbent assay with a chemiluminescent substrate (CorPlex Human Cytokine Panel 1, Quanterix, MA). All serum samples were collected in the morning (6:00 am to 8:00 am) after overnight fasting. The samples were centrifuged and stored at −80°C within 1 h after blood withdrawal. Data analysis by Wilcoxon matched-pairs signed-rank test was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, United States).

### RESULTS

#### Patient Selection and Mesenchymal Stromal Cell Characteristics

The overall results of the patient selection process are summarized in Figure 1. Of the 45 patients initially assessed for eligibility, seven patients were treated. The results of cell manufacturing in terms of MSC characteristics are shown in Table 1. The main reason for failure in obtaining a conform final product was insufficient cell growth and therefore it was unjustified to repeat the production since the same growing capacity is expected from starting material obtained from the same subject.

#### Cell Infusion

The results of the angiography performed just before cell injection are reported in Table 2. The median duration of the arterial catheterization procedure for cell infusion was 3 h and 35 min (n = 7; range: 3–4 h and 45 min). The preliminary angiographic study before cell infusion documented anatomic variation of the Willis circle in three of the seven patients. In one patient (Case 3), the right posterior cerebral artery originated directly from the carotid siphon. In this patient, the left carotid artery was extremely winding and therefore, MSCs were not infused into the left side of the cerebral circulation. No severe adverse events were registered during the infusion procedure, and the most common side effect was hematoma at the injection site.

#### Safety and Efficacy Evaluation

Regarding safety, the overall results are shown in Table 1. Single patient's data are reported in Supplementary Table 1. In addition

| TABLE 1 | Demographic, MSC characteristics, and clinical assessment data. |
|----------|---------------------------------------------------------------|
| **Demographic data** | |
| Gender (male/female) | 2/6 |
| Age (years) | 65 (60–68) |
| Disease duration (months) | 48 (36–108) |
| MSC dosage and purity | |
| Total MSCs (×10⁶) | 97.0 (77–156) |
| Cell dose (×10⁶)/kg | 1.6 (1.0–2.0) |
| Purity | 98.8 (95.3–98.9) |
| **Survival** | |
| Alive/total treated after 1 year from treatment | 6/7 |
| **Adverse events** | |
| Number of adverse events (N = 8) | 17 |
| Hematoma at the injection site | 7 |
| Asymptomatic spotty lesion | 6 |
| Left hemiparesis | 1 |
| Urticaria | 1 |
| Phosphenes | 1 |
| Hallucinosis | 1 |
| **MMSE** | |
| Baseline (n = 8) | 25.51 (24.27–28.27) |
| 1-month (n = 6) | 26.49 (24.27–28.53) |
| 12-month (n = 4) | 24.79 (21.27–25.2) |
| **H&Y score ≥ 4** | |
| Baseline (n = 8) | 8 |
| 1-month (n = 6) | 6 |
| 3-month (n = 7) | 6 |
| 6-month (n = 4) | 4 |
| 12-month (n = 4) | 4 |
| **UPDRS III** | |
| Baseline (n = 8) | 44.5 (31–58) |
| 1-month (n = 6) | 36.5 (30–48) |
| 3-month (n = 7) | 48 (27–55) |
| 6-month (n = 4) | 42.5 (39–51) |
| 12-month (n = 4) | 47 (40–49) |
| **PSP-RS** | |
| Baseline (n = 8) | 46 (29–57) |
| 1-month (n = 6) | 40 (21–48) |
| 3-month (n = 7) | 44 (24–69) |
| 6-month (n = 4) | 49.5 (31–63) |
| 12-month (n = 4) | 50 (41–57) |

Data are reported as median (range). The purity of the MSCs was calculated as a percentage of CD45−/90 + /165 + cells, MSCs, mesenchymal stromal cells; MMSE, Mini-Mental State Evaluation; H&Y, Hoehn & Yahr stage; UPDRS III, Unified Parkinson’s Disease Rating Scale part III; PSP-RS, Progressive Supranuclear Palsy Rating Scale.

1www.graphpad.com
TABLE 2 | Angiographic and post-infusion BMR data (n = 7).

| Patient | Angiography | Number of spotty lesions | Site |
|---------|-------------|--------------------------|------|
| Case 1  | Normal      | 5                        | Insula; right superior frontal and parietal cortex, left pedunculus, left peritrigonal region |
| Case 2  | Absence of A1 trait of the right anterior cerebral artery | 3 | Temporo-mesial region, optic tract, right frontal cortical–subcortical junction |
| Case 3  | Fetal origin of the right posterior cerebral artery that directly originated from carotid siphon. Tortuosity of the left carotid artery | 7 | Left cerebellar hemisphere, right pons, bilateral frontal cortex, right temporal cortex, left posterior region of the lenticular nucleus, left parietal and parasagittal cortex |
| Case 4  | Hypoplasia of A1 tract of right anterior cerebral artery | 2 | Pons and mesencephalus |
| Case 5  | Normal      | 2                        | Right subcortical temporal region, right cortical parietal region |
| Case 6  | Normal      | 3                        | Right subcortical temporal region, right cortical frontal and parietal regions |
| Case 7  | Normal      | 0                        | None |

TABLE 3 | Posturographic data.

| Posturography measurement | Healthy controls (N = 6) | Case 6 | Case 7 | Case 8 |
|---------------------------|--------------------------|--------|--------|--------|
|                           | Baseline | 6 month | Baseline | Baseline |
| Gender (F/M)              | 4/2      | F       | F       | M      |
| Age (years)               | 66 (61–69) | 66      | 66      | 65     |
| Body height (cm)          | 160.0 (147.0–176.1) | 154.4  | 154.4  | 165.9  |
| Foot length (cm)          | 24.1 (21.5–26.0) | 23.3   | 23.3   | 25.1   |
| Body weight (kg)          | 59.9 (55.0–70.0) | 76.6* | 76.6* | 66.6   |
| Base of support (cm²)     | 680.6 (503.6–736.8) | 816.9* | 1021.3* | 958.4* |
| Base width (cm)           | 14.2 (11.8–20.4) | 28.4* | 31.9* | 26.3* |
| Foot alignment (cm)       | 0.8 (0.4–1.6) | 2.4* | 5.0* | 1.8* |
| Distance CoP heel (%FL)   | 41 (34–47) | 38     | 45     | 30*    |
| Delta forces (%FL)        | 12.4 (14.1–56.1) | 21.0   | 8.5    | 83.5*  |
| Ellipse area (mm²)        | 81 (24–265) | 213    | 293*   | 422*   |
| Axis a ellipse (mm)       | 6.8 (4.4–13.7) | 13.1   | 22.8*  | 30.5*  |
| Axis b ellipse (mm)       | 3.4 (1.7–6.7) | 5.2    | 4.1    | 4.4    |
| Eccentricity              | 0.89 (0.80–0.95) | 0.89*  | 0.98*  | 0.99*  |
| Mean distance (mm)        | 3.4 (2.0–6.5) | 6.3    | 9.8*   | 13.3*  |
| RMS distance (mm)         | 3.9 (2.4–7.5) | 7.2    | 11.9*  | 15.7*  |
| Median frequency (Hz)     | 0.5 (0.3–0.6) | 0.4    | 0.3*   | 0.2*   |

Data are shown as the median (range) for the healthy control group and as the median of at least three assessments for the patients. *Data below/above the 10th/90th percentile of the distribution of healthy controls.

to the previously published results (Canesi et al., 2016), the two patients treated in the second phase were living 1 year after the procedure. For one patient (Case 6), the following three adverse events were registered after cell infusion. (1) Phosphenes were observed before and during the catheterization procedure. (2) Millimeter-scale ischemic alterations in BMR signals were observed after the catheterization procedure. These lesions were not observed in the BMR 2 weeks later. (3) The patient experienced hallucinosis and reported perceiving clear images of animals or persons on a white wall, especially at twilight. This phenomenon occurred more than 48 h after cell infusion and lasted less than 12 h. No further adverse events were reported for this subject or the other patient.

The day after cell infusion, BMR showed spotty signal alterations compatible with recent ischemic lesions in one of the two patients treated in the second phase (overall six of the seven treated patients). This patient was asymptomatic. As previously reported (Canesi et al., 2016), spotty lesions were observed in the last patient of the first cohort. In addition, this patient showed ischemic alterations in the posterior segment of the left inferior peduncle of the cerebellum and in the right mesencephalon, and these symptoms were transient.

A summary of our clinical findings for efficacy is shown in Table 1 and Figure 2. Single patient’s data are also contained in Supplementary Table 1. Notably, one patient (Case 6) had an improved UPDRS III score 3 months after cell infusion.
Inflammatory Cytokine Measurements

Cytokine analysis was performed on all of the treated patients ($n = 7$; Supplementary Figure 1). The differences in cytokine serum concentrations before and after cell administration were not statistically significant (Wilcoxon matched-pairs signed-rank test). In three of the seven patients (Case 2, 3, and 4), the values of IL-8 (pro-inflammatory) were more than five times lower than those in the basal samples after cell administration (4.01, 6.66, and 25.09%, respectively).

DISCUSSION/CONCLUSION

In 2016 we already published the clinical results obtained in the first exploratory phase of our clinical protocol based on the use of MSCs in five patients affected by PSP (Canesi et al., 2016). We were therefore authorized by our competent
and ineffective. Consequently, we would not have been able to
make a decision primarily because the recruitment process was slow
and independent from the cells and others potentially linked
to the still controversial pro-coagulant effects of MSC (Coppin
et al., 2019). In the previous clinical experience with intra-
arterial infusion of MSC for multiple systemic atrophy reported
by Lee et al. (2012), microembolization was indeed present
also in the placebo group and at a higher frequency compared
to the treated group (35% vs 29%). Other factors that may
be involved in microembolization/microbleeding during MSC
intra-arterial administration are cell size and type (Kar lupia
et al., 2014), and infusion velocity (Cui et al., 2015). Also, the
effect of heparin or of excipients in the final product may be
involved, even though no clear evidence is reported to date.
Notable, DMSO has been proven to have some toxicity on red
blood cells, platelet, and vascular endothelial cells also at a
concentration below 1% (Yi et al., 2017). The causal relationship
between all these factors and the reported adverse events remains
to be established. From the clinical point of view, it cannot
remain unsaid that what we defined as asymptomatic lesions as
so far as we know, may have some less obvious negative impact
on the clinical progression. As a conclusion, the risk/benefit
balance of the approach followed in our trial has still to be
improved. Regarding efficacy, unfortunately, only four of the
seven treated patients were able to complete the 1-year follow-
up. One patient died after an accidental fall 4 months after
treatment, and two patients were not compliant to longer follow-
up. In all of the four patients who completed follow-ups, we
observed stabilization on at least two clinical scales until 6
months after cell infusion. In two patients, the stabilization
threshold was met on only the UPDRS-III or PSP-RS. No
variation was documented for PET. The overall safety and
efficacy results are still inconclusive at this time because of the
low number of patients and consequently the poor statistical
power of the study.

At the end of the second part of the pilot phase, we
decided not to proceed to the randomized phase. We made this
decision primarily because the recruitment process was slow
and ineffective. Consequently, we would not have been able to
reach definitive conclusions regarding the risk/benefit ratio of the
proposed approach in a reasonable period.

As discussed in our previous research (Giordano et al., 2014),
MSCs may aid in the treatment of neurodegenerative disorders
by restoring neural cell function and homeostasis and by exerting
anti-apoptotic and anti-inflammatory effects on neural and glial
cells, thus reducing neuroinflammation. Despite having a very
promising mechanism of action, cell therapy for complex and
rare diseases like PSP carries several intrinsic limitations, which
affected our work. In particular, we highlight the difficulty in
identifying the best candidates for MSC treatment among
patients that suffer from heterogeneous and rapidly progressive
disorders, such as PSP.

In spite of these challenges, the experience gained in our trial
is valuable for the design, planning, and management of future
clinical trials. Indeed, finding novel therapeutic approaches for
orphan diseases remains a priority and cell-based regenerative
therapies represent a promising tool in this field (Tambuyzer
et al., 2020). Among other cell types, MSCs have dramatic anti-
inflammatory and immunomodulatory properties (Marigo and
Dazzi, 2011). Moreover, an inflammatory environment is an
important determinant of the efficacy of cellular therapy (van
Megen et al., 2019). Interestingly, in our study, two of the three
patients (Cases 2 and 3) who showed significant reductions
in serum IL-8 concentrations also had improvements in both
UPDRS and PSP-RS at the 1-month follow-up after treatment,
and they showed improvements in specific symptoms (neck pain,
ocular motility, and photophobia). These results were previously
published (Canesi et al., 2016). Due to the insufficient sample
size, we cannot consider these results as more than anecdotal
events, but the short-term effect of cell-based therapies on
specific symptoms should be addressed in further studies. In
addition, it will be important to examine the effect of repeated
cell administration.

Another important issue for the development of phase II
efficacy studies is applying the right diagnostic classifications for
suitable patients. The Movement Disorder Society (MDS)
has recently identified diagnostic criteria for PSP (Höglinger
et al., 2017) and defined eight predominance types: PSP-
RS, Richardson syndrome; PSP-PI, predominant postural
instability; PSP-OM, predominant ocular motor dysfunction;
PSP-P, predominant parkinsonism; PSP-PG, progressive gait
freezing; PSP-CBS, predominant corticobasal syndrome; PSP-F,
predominant frontal presentation, and PSP-SL, predominant
speech/language disorder. Notably, all of the patients enrolled
in our study were classified as having Richardson’s syndrome.
The correct classification of the patients in a context of a clinical
trial is not a trivial problem and it can affect the reliability of
efficacy evaluation. That’s why, to overcome the problem of
multiple diagnostic allocations of a single patient, guidelines
are now available for the application of the new criteria in PSP
patients who show symptoms of more than one functional
domain (so-called MAX rules for multiple allocation extinction;
Grimm et al., 2019). The MDS criteria for PSP also introduced
the category “probable 4-repeat (4R)-tauopathy” for joint clinical
diagnosis of PSP and corticobasal degeneration. These new
diagnostic categories and classifications have high specificity and
may be suitable for the recruitment of patients with PSP and corticobasal degeneration into therapeutic trials.

The scarcity of validated in vivo disease-specific biomarkers for both the diagnosis and the assessment of disease progression is another important bottleneck for early phase interventional trials in PSP. To improve the reliability of our results, we used multiple clinical scales and empirically considered longitudinal changes of ±30% as significant in terms of effectiveness. This cut-off value was defined by comparisons with cohorts of patients with the same characteristics (Litvan and Kong, 2014). We also performed a detailed biomechanical evaluation and state-of-the-art imaging studies with the aim of documenting the rate of change after the experimental treatment. For future efficacy trials, in vivo imaging biomarkers (e.g., Tau-PET) as well as multiple CSF and blood biomarkers should be considered.

Our study revealed critical considerations in cell manufacturing when using autologous cells from aged and diseased subjects. In our experience, 8 out of the 12 treatment failures (67%) were due to cell-related causes (low numbers or non-compliant quality controls). We have previously demonstrated that several disease-related factors affect the cell growth potential of MSCs from PSP patients (e.g., microtubule instability may lead to altered paracrine function and mitochondrial dysfunction may lead to altered cell growth; Angelova et al., 2018; Calogero et al., 2018). These factors may explain the deviations we recorded in cell production.

In performing this phase I study, we identified several critical issues in the production and use of ATMPs for rare diseases in autologous settings. This work encourages further studies using innovative designs (e.g., adaptive studies), employing allogenic cells as starting material and a-cellular therapy approaches. In addition, our experience supports the use of technological innovations for safer cell delivery.

In conclusion, the main question addressed in our work is “How can we safely treat neurodegeneration with cell therapy”? Indeed, the anti-inflammatory properties of MSCs are at the moment exploited in many pathological contexts, but they are still poorly applied in neurodegenerative disorders. In particular, at the date of submission of the present article, there is only one study registered at clinicaltrials.gov using BM-derived MSCs for the treatment of several neurologic diseases including PSP (ClinicalTrials.gov Identifier: NCT02795052) and on June 1, 2021, a search in Pubmed using the key-words “Progressive Supranuclear Palsy,” “Parkinsonisms,” “Cell,” and “Clinical trial” gives rise to 10 articles and only two of them are pertinent, both from our study group. Therefore, it is clear that the many bottlenecks and concerns that we have herein underlined discourage researchers to study the clinical effects of cell therapy in neurodegenerative disorders. Our pioneeristic experience may be of great help for people working in the search of advanced therapeutic tools for still unmet medical needs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico (Italy). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RG and MC made substantial contributions to the conception, organization, and execution of this work; to the writing of the manuscript and to its review and critique. Specifically, RG established good manufacturing practices (GMP) procedures for MSCs including validation, production, and quality controls; wrote protocols; submitted the trial to regulatory authorities; and coordinated the project. MC performed patient selection, clinical evaluations, and follow-up evaluations. MI performed cell administration. II and CP performed multifactorial movement analysis and analyzed the results. II aided in revising the manuscript. RB and GiorM defined and performed the PET/SPECT procedures and analyzed the results. GiovM and RC performed clinical assessments of the patients before, during, and after treatment and described and reported all adverse events. AR and VC performed neuropsychological assessments. LL, TM, and MV aided in establishing GMP for MSC, including validation, production, and quality controls. SB helped prepare and submit the trial to regulatory authorities and collected patient data during the study follow-up. EM and CL contributed to MSC production and quality control. DV performed bone marrow aspiration and hematological patient assessments. AR-O performed cytokine studies, interpreted data, and helped revise the manuscript. GP made substantial contributions to the conception, design, organization, and execution of this research project. Specifically, GP conceived the clinical trial, contributed to the study design, and aided in writing the research protocol. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.723227/full#supplementary-material

**REFERENCES**

Angelova, P. R., Barilani, M., Lovejoy, C., Dossena, M., Viganò, M., Seresini, A., et al. (2018). Mitochondrial dysfunction in Parkinsonian mesenchymal stem cells impairs differentiation. *Redox Biol.* 14, 474–484. doi: 10.1016/j.redox.2017.10.016

Bevan-Jones, W. R., Cope, T. E., Jones, P. S., Passamonti, L., Hong, Y. T., Fryer, T., et al. (2019). In vivo evidence for pre-symptomatic neuroinflammation in a MAPT mutation carrier. *Ann. Clin. Transl. Neurol.* 6, 373–378. doi: 10.1002/acn3.683

Brazzini, A., Cantella, R., De la Cruz, A., Yupaquji, J., León, C., Jorquiera, T., et al. (2010). Intraarterial autologous implantation of adult stem cells for patients with Parkinson disease. *J. Vasc. Interw. Radiol.* 21, 443–451. doi: 10.1016/j.jvir.2010.01.008

Calogero, A. M., Viganò, M., Budelli, S., Galimberti, D., Fenoglio, C., Cartelli, D., et al. (2018). Microtubule defects in mesenchymal stromal cells distinguish patients with progressive supranuclear palsy. *J. Cellular Mol. Med.* 22, 2670–2679. doi: 10.1111/jcm.13545

Canesi, M., Giordano, R., Lazzari, L., Isalberti, M., Isaias, I. U., Benti, R., et al. (2016). Finding a new therapeutic approach for no-option Parkinsonisms: mesenchymal stromal cells for progressive supranuclear palsy. *J. Transl. Med.* 14:127.

Coppin, L., Sokal, E., and Stéphenne, X. (2019). Thrombogenic risk induced by intravascular mesenchymal stem cell therapy: current status and future perspectives. *Cells* 8:1160. doi: 10.3390/cells8111160

Cui, L., Kerkela, E., Bakreen, A., Nitzsche, F., Andrzejewska, A., and Nowakowski, K. (2020). The neutrophil-to-lymphocyte ratio as a marker of peripheral inflammation in progressive supranuclear palsy: a retrospective study. *Neurosci. 41, 1233–1237.* doi: 10.1016/s0072-019-04208-4

Cyranoski, D. (2012). Canada approves stem cell product. *Nat. Biotechnol.* 30:571. doi: 10.1038/nbt0712-571b

De Becker, A., and Riet, I. V. (2016). Homing and migration of mesenchymal stromal cells: how to improve the efficacy of cell therapy? *World J. Stem Cells* 8, 73–87. doi: 10.4252/wjsc.v8.i3.73

Dickson, D., Hazw, I., Azid, Y., and Litvan, I. (2011). “Progressive supranuclear palsy and corticobasal degeneration,” in *Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders*, 2nd Edn, eds D. W. Dickson and R. O. Weller (Oxford: Wiley-Blackwell), 135–155.

Eveeraet, B. R., Bergwerf, I., De Vocht, N., Ponsaerts, P., Van Der Linden, A., Timmermans, J. P., et al. (2012). Multimodal in vivo imaging reveals limited allograft survival, intrapulmonary cell trapping and minimal evidence for ischemia-directed BMSC homing. *BMC Biotechnol.* 12:93. doi: 10.1186/1427-6750-12-93

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). “Mini-mental state.” A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.

Gay, M. H. P., Baldomero, H., Farge-Bancel, D., Robey, P. G., Rodeo, S., Passweg, J., et al. (2021). The survey on cellular and tissue-engineered therapies in Europe in 2016 and 2017. *Tissue Eng. Part A 27, 336–350.* doi: 10.1089/ten.tea.2020.0092

Giordano, R., Canesi, M., Isalberti, M., Isaias, I. U., Montemurro, T., Viganò, M., et al. (2014). Autologous mesenchymal stem cell therapy for progressive supranuclear palsy: translation into a phase I controlled, randomized clinical study. *J. Transl. Med.* 12:14. doi: 10.1186/1479-8676-12-14

Goetz, C. G., Poeoe, W., Rascol, O., Sampaio, C., Stobbins, G. T., Counsell, C., et al. (2004). Movement Disorder Society Task Force report on the Hoehn and Yahr staging scale: status and recommendations. *Mov. Disord.* 19, 1020–1028. doi: 10.1010/520213

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stobbins, G. T., Fahn, S., Martinez-Martin, P., et al. (2008). Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* 23, 2129–2170. doi: 10.1002/mds.22340

Golbe, L. I., and Ohman-Strickland, P. A. (2007). A clinical rating scale for progressive supranuclear palsy. *Brain 130, 1552–1565.* doi: 10.1093/brain/awn032

Grimm, M. J., Respondek, G., Stamelou, M., Arzberger, T., Ferguson, L., Geldpi, E., et al. (2019). Movement Disorder Society-endorsed PSP Study Group. How to apply the movement disorder society criteria for diagnosis of progressive supranuclear palsy. *Mov. Disord. 34, 1228–1232.* doi: 10.1002/mds.27666

Höglinger, G. U., Respondek, G., Stamelou, M., Kurz, C., Josephs, K. A., Lang, A. E., et al. (2017). Movement Disorder Society-endorsed PSP Study Group. Clinical diagnosis of progressive supranuclear palsy: the movement disorder society criteria. *Mov. Disord. 32, 853–864.* doi: 10.1002/mds.26987

Inci, I., Kushbe, O. Y., and Eskut, N. (2020). The neutrophil-to-lymphocyte ratio as a marker of peripheral inflammation in progressive supranuclear palsy: a retrospective study. *Neurosci. 41, 1233–1237.* doi: 10.1016/s10072-019-04208-4

Karlapia, N., Manley, N. C., Prasad, K., Schäfer, R., and Steinberg, G. K. (2014). Intraarterial transplantation of human umbilical cord blood mononuclear cells is more efficacious and safer compared with umbilical cord mesenchymal stromal cells in a rodent stroke model. *Stem Cell Res. Ther.* 5, 45–63.

Lee, P. H., Lee, J. E., Kim, H. S., Song, S. K., Lee, H. S., Nam, H. S., et al. (2012). A phase IIa, double-blind, placebo-controlled randomized clinical trial of mesenchymal stem cells in patients with progressive supranuclear palsy. *J. Psychiatr. Res.* 46, 11–19. doi: 10.1016/j.jpsychires.2011.09.004

Littvin, I., and Kong, M. (2014). Rate of decline in progressive supranuclear palsy. *Mov. Disord.* 29, 463–468. doi: 10.1002/mds.25843

Malpetti, M., Passamonti, L., Jones, P. S., Street, D., Rittman, T., Fryer, T. D., et al. (2021). Neuroinflammation predicts disease progression in progressive supranuclear palsy. *J. Neurosurg. Psychiatry* 92, 769–775. doi: 10.1136/jnp-psychiatry-2020-325549

Marigo, I., and Dazzi, F. (2011). The immunomodulatory properties of mesenchymal stem cells. *Semin. Immunopathol.* 33, 593–602.

Montemurro, T., Viganò, M., Budelli, S., Montelatici, E., Lavarza, C., Marino, L., et al. (2015). How we make cell therapy in Italy. *Drug Des. Devel. Ther.* 9, 4825–4834. doi: 10.2147/dddt.s88403

Palmisano, C., Todisco, M., Marotta, G., Volkman, J., Pacchetti, C., Frigo, C. A., et al. (2020). Gait initiation in progressive supranuclear palsy: brain metabolic correlates. *Neuroimage Clin.* 28:102408. doi: 10.1016/j.nicl.2020.102408
Paviour, D. C., Price, S. L., Jahanshahi, M., Lees, A. J., and Fox, N. C. (2006). Regional brain volumes distinguish PSP, MSA-P, and PD: MRI-based clinico-radiological correlations. Mov. Disord. 21, 989–996.

Sensebe, L., and Fleury-Cappellesso, S. (2013). Biodistribution of mesenchymal stem/stromal cells in a preclinical setting. Stem Cells Int. 2013:678063. doi: 10.1155/2013/678063

Steele, J. C., Richardson, J. C., and Olszewski, J. (1964). Progressive supranuclear palsy: a heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. Arch Neurol. 10, 333–359. doi: 10.1001/archneur.1964.00460160003001

Tambuyzer, E., Vandendriessche, B., Austin, C. P., Brooks, P. J., Larsson, K., Needleman, K. I. M., et al. (2020). Therapies for rare diseases: therapeutic modalities, progress and challenges ahead. Nat. Rev. Drug Discov. 19, 93–111. doi: 10.1038/s41573-019-0049-9

van Megen, K. M., van ‘t Wout, E.-J. T., Lages Motta, J., Dekker, B., Nikolic, T., and Roep, B. O. (2019). Activated mesenchymal stromal cells process and present antigens regulating adaptive immunity. Front. Immunol. 10:694. doi: 10.3389/fimmu.2019.00694

Yi, X., Liu, M., Luo, Q., Zhuo, H., Cao, H., Wang, J., et al. (2017). Toxic effects of dimethyl sulfoxide on red blood cells, platelets, and vascular endothelial cells in vitro. FEBS Open Bio. 7, 485–494. doi: 10.1002/2211-5463.12193

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