Final Results of Allogeneic Adipose Tissue–Derived Mesenchymal Stem Cells in Acute Ischemic Stroke (AMASCIS): A Phase II, Randomized, Double-Blind, Placebo-Controlled, Single-Center, Pilot Clinical Trial

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Abstract

Acute ischemic stroke is currently a major cause of disability despite improvement in recanalization therapies. Stem cells represent a promising innovative strategy focused on reduction of neurologic sequelae by enhancement of brain plasticity. We performed a phase IIa, randomized, double-blind, placebo-controlled, single-center, pilot clinical trial. Patients aged ≥60 years with moderate to severe stroke (National Institutes of Health Stroke Scale [NIHSS] 8–20) were randomized (1:1) to receive intravenous adipose tissue–derived mesenchymal stem cells (AD-MSCs) or placebo within the first 2 weeks of stroke onset. The primary outcome was safety, evaluating adverse events (AEs), neurologic and systemic complications, and tumor development. The secondary outcome evaluated treatment efficacy by measuring modified Rankin Scale (mRS), NIHSS, infarct size, and blood biomarkers. We report the final trial results after 24 months of follow-up. Recruitment began in December 2014 and stopped in December 2017 after 19 of 20 planned patients were included. Six patients did not receive study treatment: two due to technical issues and four for acquiring exclusion criteria after randomization. The final study sample was composed of 13 patients (4 receiving AD-MSCs and 9 placebo). One patient in the placebo group died within the first week after study treatment delivery due to sepsis. Two non-treatment-related serious AEs occurred in the AD-MSC group and nine in the placebo group. The total number of AEs and systemic or neurologic complications was similar between the study groups. No injection-related AEs were registered, nor tumor development. At 24 months of follow-up, patients in the AD-MSC group showed a nonsignificantly lower median NIHSS score (interquartile range, 3 [3–5.5] vs 7 [0–8]). Neither treatment group had differences in mRS scores throughout follow-up visits up to month 24. Therefore, intravenous treatment with AD-MSCs within the first 2 weeks from ischemic stroke was safe at 24 months of follow-up.

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Submitted: November 10, 2021. Revised: January 31, 2022. Accepted: February 11, 2022.

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Introduction

Stroke is one of the most common causes of mortality worldwide and is a leading cause of disability. The estimated global incidence of stroke in 2016 was found to be between 189 and 218 cases per 100,000 inhabitants[1]. In the last two decades, vast improvements have been made in treatments for ischemic stroke, such as intravenous thrombolysis and mechanical thrombectomy. However, many patients suffer persistent neurological deficits and systemic complications. Current treatments are focused on reestablishing cerebral perfusion in the acute phase of stroke (usually limiting their applicability to the first 6–12 h from symptom onset), but do not favor regeneration once brain tissue is damaged. There is an urgent need for more widely available therapies focused on cerebral tissue repair, which would complement the current therapeutic options, extend the treatment time window, and help mitigate the disability and burden from stroke.

Cell therapies are innovative treatments that enhance brain tissue repair after ischemic damage through stimulation of neurogenesis, gliogenesis, oligodendrogenesis, synaptogenesis, and angiogenesis[2]. Stem cells are immature cells able to differentiate into diverse cell lines, which have also shown the capacity to secrete trophic factors and modulate the immune response, improving functional recovery after stroke in preclinical studies[3,4]. Although cell therapy in preclinical studies has shown beneficial effects, current challenges include finding suitable cell types, the best route of administration, and the timing and dose before translation to clinical practice is feasible[3–6].

Many cell types have been used in preclinical stroke studies and clinical trials (eg, mononuclear cells[7–9], neural stem cells, embryonic stem cells, induced pluripotent stem cells, mesenchymal cells)[3,10–14]. Among them, mesenchymal stem cells (MSCs) appear to be safe, possibly effective, and have advantages for clinical applicability because they are easy to obtain, have relatively low immunogenicity, and lack bioethical concerns. MSCs can derive from various sources, such as bone marrow, adipose tissue, dental pulp, and umbilical cord tissue. Adipose-derived MSCs in particular have the advantage of being abundant and easy to obtain by lipoaspiration techniques. Concerning mesenchymal cell origin, although autologous stem cells could initially appear safer, allogeneic MSCs lack human leucocyte antigen class II molecules, thus avoiding the risk of rejection. They also allow the possibility of having a cell bank readily available, which would permit earlier treatment administration.

Regarding the route of delivery, cell-based therapies have been dispensed by direct implantation into the central nervous system (intracerebral, intraventricular, or subarachnoid transplantation) based on the erroneous concept that stem cells' basic mechanism of action was replacement of damaged brain tissue cells. However, less invasive systemic routes, such as intra-arterial, intravenous, and intranasal, are gaining more importance because the paracrine action of these cells is believed to play the primary role in brain regeneration. Preclinical studies have demonstrated that the intravenous infusion of stem cells is noninferior to other more invasive routes[3,15], making this a propitious delivery route for translation to clinical trials.

In preclinical studies, cell therapy has typically been administered in the acute phase of stroke (within 24 h from symptom onset), with promising safety and efficacy results[10–12]. However, most clinical trials are performed in later phases of stroke when stem cells can only act on repair but not on brain protection mechanisms, with poor efficacy results, albeit no safety concerns[6]. The scarce trials in which an early treatment window has been explored have shown similar safety results, although trends toward a better functional status have been suggested[7,8].

Cell dosages vary in animal models of stroke, at between 10^4 and 4.3×10^7 cells per kilogram of weight. Results appear to be more beneficial for lower rather than higher doses of MSCs, which could be due to a potential embolus formed with a higher dosage or slowing of cerebral blood flow[10–13]. In clinical trials, there is no standard dosage. The recommended dose is 1 to 2×10^6 cells per kilogram, although higher doses have been used without safety concerns.

Intravenous infusion of adipose-derived mesenchymal stem cells (AD-MSCs) has obtained good results in rat models of ischemic stroke. No safety issues have been observed, and positive results in functional recovery, reduction of brain tissue cell death, and increased cell proliferation in the peri-infarct zone have been described[16–18]. Currently, the RESSTORE 1 phase Ia/Ib clinical trial is recruiting patients with hemispheric nonlacunar stroke to be treated with various doses of intravenous AD-MSC solution or placebo. Safety and efficacy results after a 2-year follow-up period, including blood biomarkers and functional magnetic resonance imaging (MRI), are not yet available.

Therefore, in the Allogeneic Adipose Tissue–Derived Mesenchymal Stem Cells In Acute Ischemic Stroke (AMASCIS) trial, our main objective was to demonstrate the safety of intravenous AD-MSC delivery within the first 2 weeks from stroke symptom onset and to explore the possible efficacy in reduction of stroke-associated disability. To our knowledge, this was the first clinical trial exploring intravenous use of AD-MSCs in patients with ischemic stroke. Preliminary safety and efficacy results after 6 months of follow-up have already been comunicated[19]; here, we present the final clinical trial results.

Materials and Methods

Study Design

The AMASCIS trial was a phase IIa, pilot, single-center, prospective, randomized, double-blind, placebo-controlled clinical trial to evaluate the safety of a single intravenous dose of 1 million AD-MSCs per kilogram of weight compared with placebo in the first 2 weeks after ischemic stroke. It was an academic trial, promoted by the Research Foundation from La Paz University Hospital, funded by the Spanish Ministry of Health and Social Policy, and approved by the La Paz University Hospital Ethics Committee and by the Spanish Agency of Medicines and Health Products in 2011 (EudraCT: 2011-003551-18). The clinical trial protocol was published before recruitment began[20] and is registered in Clinicaltrials.gov (NCT01678534).

Participants

Initially, we planned to enroll 20 patients aged 60 to 80 years with moderate to severe ischemic stroke in the region of the middle cerebral artery (MCA) with a National Institute of Health Stroke Scale (NIHSS) of 8 to 20 points, who had arrived at the hospital less than 12 h from stroke symptom onset. An initial computed tomography (CT) and/or MRI scan compatible with ischemic stroke in MCA was needed for inclusion. Patients received standard treatment for acute ischemic stroke, including recanalization therapies such as intravenous thrombolysis or mechanical thrombectomy. All patients (or their legal representative or relative) signed the informed consent document after being given a detailed explanation of the nature and purpose of this study and prior to the performance of any of the study-related procedures. Patients in a comatose state (score ≥ 2 on item 1a of the NIHSS); evidence of brain tumor, primary intraventricular, intracerebral, or subarachnoid hemorrhage on neuroimaging; cerebral edema with midline shift; or cerebellar or brainstem infarction were excluded. Clinical or laboratory signs suggestive of active infection, including human immunodeficiency virus and hepatitis B and C, were also considered exclusion criteria, along with a previous diagnosis of dementia or any health condition that could preclude appropriate diagnosis, treatment, or follow-up.

Recruitment began in March 2014, but due to inclusion difficulties, a protocol amendment was made to eliminate the upper age limit and to lengthen the time of arrival at the hospital to 24 h. The complete inclusion and exclusion list is included as supplemental material. This first amendment was approved by the ethics committee in December 2014, just after the first patient was enrolled. A second protocol amendment was approved in April 2016 to update information on standard acute ischemic stroke treatment, without inclusion and exclusion criteria modification.

Randomization and Masking

Each patient was assigned a unique number as they were enrolled in the study and was then allocated by a study nurse (nonblinded to the study treatment) to one of the two study groups (AD-MSCs or placebo) in a 1:1 ratio according to a computer-generated, simple randomization list prepared by an independent statistician. The supply for the study drug was then ordered by the same nurse from the cell's manufacturer. After the study treatment was received at the Clinical Trials Unit, it was labeled with a unique identification number. To maintain blinding, vials containing placebo and AD-MSCs were identical and protocols were designed to ensure that physicians evaluating patient safety and efficacy outcomes would not have access to randomization codes.

Procedures

The experimental drug was a solution of allogeneic AD-MSCs, obtained by lipoaspiration techniques from a healthy donor, cultured *in vitro*, packed in cryovials with dimethyl sulfoxide and fetal bovine serum, and cryopreserved at −180°C as instructed by the manufacturer Histocell S.L. (Bizkaia, Spain). Before use, cryovials were thawed, washed, and finally packed in vials containing only cell product together with glucosaline solution, sodium bicarbonate, human albumin, and Ringer's lactate. Details concerning the manufacturing processes and quality controls are specified in the investigator's brochure, which is available at the study center and has been approved by the Spanish Agency of Medicines and Health Products.

The final experimental drug solution was composed of 10 million cells per milliliter of solution to be administered intravenously at a dose of 1 million cells per kilogram of the patient's weight (0.1 ml per kilogram of the patient's weight in the case of placebo solution) within 2 weeks from stroke symptom onset. All patients received conventional treatment for ischemic stroke according to the valid guidelines at that time.

The total study duration was 24 months and consisted of 10 scheduled patient visits (V0, screening; V1, pretreatment; V2, 2 h after treatment; V3, 24 h after treatment; V4, day 7 after treatment; V5, month 3; V6, month 6; V7, month 12; V8, month 18; V9, month 24).

Outcomes

The study's primary outcome was safety, and it was assessed at each visit by evaluating adverse events (AEs) reported spontaneously or in response to prespecified questions at each visit, such as neurologic and systemic complications (deteriorating stroke, stroke recurrences, brain edema, seizures, hemorrhagic transformation, respiratory infections, urinary tract infections, deep vein thrombosis, pulmonary embolism, and gastrointestinal hemorrhage) and tumor development. Serious AEs (SAEs) were recorded at each

visit and were immediately reported, following good clinical practice.

Secondary outcomes were included to explore the efficacy of the study treatment by using clinical parameters and neuroimaging. The modified Rankin Scale (mRS) was measured at baseline as well as at day 7 and month 3, whereas the NIHSS scale was measured at every scheduled visit. An mRS score of 0–2 and/or an NIHSS score of 0–1 were considered successful. Additional MRIs were performed at day 7 and month 3 to measure infarct size. An exploratory analysis was also performed to assess the influence of AD-MSC administration on serum levels of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP9), and extracellular vesicles (EVs). These biomarkers were collected before treatment administration and at 7 days and 3 months after. Blood was drawn from the antecubital vein, collected in BD Vacutainer Plus Plastic Serum tubes, and then centrifugated at $3,000 \times g$ for 15 min. The serum levels of BDNF, VEGF, and MMP-9 were measured by enzyme-linked immunosorbent assay (ELISA), using protocols supplied by the manufacturer. EVs were isolated from the patient's serum with an EV isolation kit (ExoQuick Ultra EV precipitation solution; System Biosciences, Palo Alto, CA, USA), following the manufacturer's instructions. After isolation, EV levels from 1 ml of serum were analyzed by the EXOEL-CD63A-1 ELISA kit (System Biosciences), using protocols supplied by the manufacturer. Commercially available ELISA kits for measurement of all biomarkers were employed by an investigator who had no knowledge of the patients' treatment allocation.

Sample Size Calculation, Statistical Analysis, and Data Management

The AMASCIS trial aimed to recruit 20 patients. A formal predetermination of sample size was not calculated because of the exploratory nature of this pilot study. This sample size should suffice to obtain enough information for the development of future phase IIb or III clinical trials according to the CONSORT (CONsolidated Standards of Reporting Trials) 2010 statement for the extension to randomized pilot and feasibility trials[21].

Demographic and clinical characteristics were summarized in terms of median and interquartile range (IQR) or percentages as appropriate for each variable type. Due to the limited sample size, a univariate approach was employed for calculating nonparametric tests for the continuous variables (Wilcoxon rank-sum) and Fisher's or chi-squared tests for the categorical variables. Only *P* values less than 0.05 were considered significant. Statistical computations were performed using SPSS version 21. The safety analysis performed for the patients who received study treatment included tables with all AEs and neurologic and systemic complications. For efficacy variables, a descriptive analysis

of the "per protocol" population was performed, as well as an exploratory analysis comparing both treatment groups.

All data management followed the principles of the Spanish data protection Law "Ley Orgánica de Protección de datos" (LOPD 15/1999) and the European regulations/ International Conference of Harmonization Good Clinical Practice guidelines. Independent Clinical Research Associates of the Clinical Trial Unit of La Paz University Hospital, not involved in any procedures, performed the data monitoring. An independent data monitoring committee periodically reviewed the unblinded data to identify any safety issues. Reports from this data monitoring committee were released in February and September 2016 and in March 2018; all of them concluded that there were no safety issues and recommended that the trial continue as planned.

Role of the Funding Source

The AMASCIS trial was an academic, investigator-initiated study funded by the Spanish Ministry of Health and Social Policy in a research call aimed to support independent study design, data collection, analysis, interpretation, and result reporting (project code EC 10/171). Most of the funding was used to acquire the study treatment from the cell manufacturer, which was not involved in any other study procedure. The corresponding authors and the writing group had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The first patient was enrolled in December 2014. Recruitment was transiently stopped several times during the summer and Christmas periods due to periodically planned qualifying works at the AD-MSC manufacturer. In addition, from January to June 2015, trial recruitment also stopped due to a relevant amendment in the manufacturing process that needed approval from the Spanish Agency for Medicines and Health Products. Recruitment was ultimately stopped on December 2017 after 19 of the 20 planned patients were included. The follow-up period of the last enrolled patient was finalized in September 2019.

Of the 19 patients initially enrolled, 6 did not receive the trial treatment (2 because of technical issues during the manufacturing process and 4 due to development of exclusion criteria after being randomized). The final study sample was composed of 13 patients: 4 received AD-MSCs and 9 received placebo solution (Fig. 1). No differences in the baseline or demographic characteristics between the treatment groups were found (Table 1). The median time from stroke to treatment administration was 13 days (IQR, 13–13.75) in the AD-MSC treatment group and 12 (IQR, 10–13) days in the placebo group ($P = 0.028$).

Regarding the primary outcome, a total of 124 AEs were reported throughout the 24 months of follow-up (complete
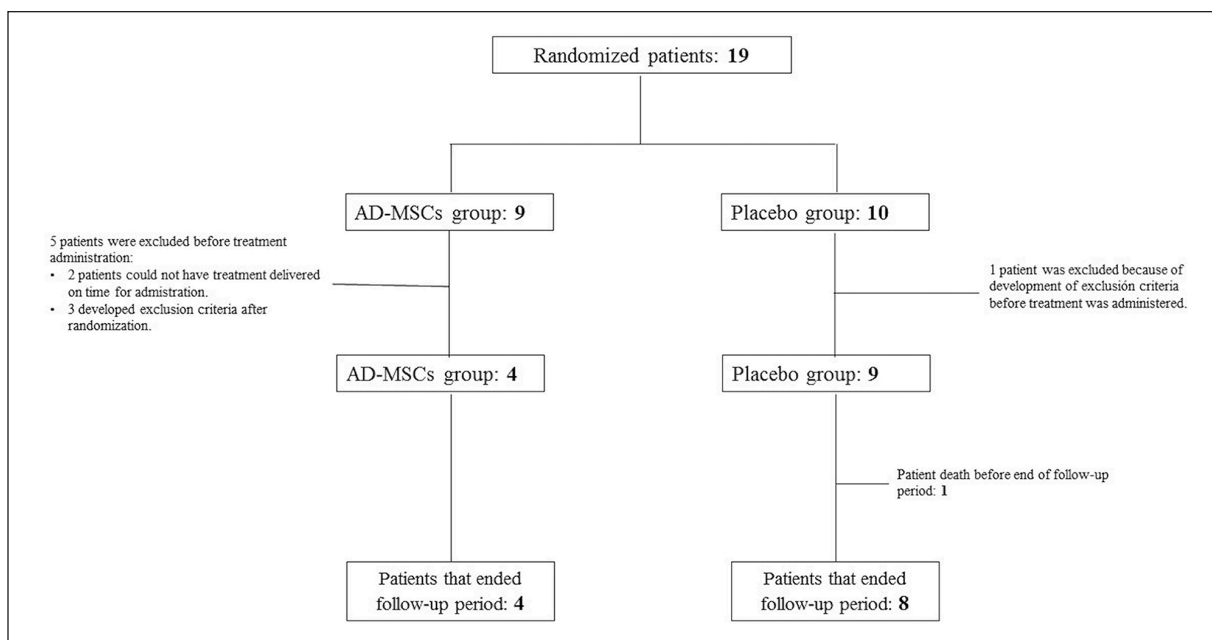
Figure 1. AMASCIS final study sample. Trial profile. Nineteen patients were initially randomized: 9 to the AD-MSC group and 10 to the placebo treatment group. In the AD-MSC group, two patients did not receive the study treatment because it could not be delivered on time for administration (2 weeks since symptom onset), and three developed exclusion criteria after randomization and before study treatment was applied. In the placebo group, only one patient developed exclusion criteria before treatment administration. Finally, AD-MSCs were administered to four participants and placebo to eight. One of these eight patients died before the end of the follow-up period. AD-MSCs: adipose tissue–derived mesenchymal stem cells; AMASCIS: Allogeneic Adipose Tissue–Derived Mesenchymal Stem Cells in Acute Ischemic Stroke.

list of AEs in supplementary material): 50 in the AD-MSC treatment group and 74 in the placebo arm ($P = 0.074$). Eleven SAEs were reported (full details provided in Table 2): two in the cell treatment arm and nine in the placebo group. The most frequent AEs were depression (present in all the patients treated with AD-MSCs and in 22.2% of the patients in the placebo arm), urinary tract infections (75% of the AD-MSC treatment group and 55.5% of the placebo group), and respiratory infections (25% of the AD-MSC group and 44.4% of the placebo group). Only one patient in the placebo treatment group died within the first 7 days of treatment administration, due to a multiorgan failure. There was no AE considered to be related to AD-MSC administration. No injection-related AEs or tumor developments were reported. No differences were found in the frequency of predefined systemic or neurological complications between the study groups (Table 3).

Results from the efficacy analysis showed no significant differences between treatment groups regarding mRS (Fig. 2), NIHSS scores, or infarct size in neuroimaging during the predefined visits for their evaluation. The analysis of the NIHSS scale during all scheduled visits showed no differences in obtaining successful scores between the treatment groups; nor were there any differences found when NIHSS score distributions were compared (Table 4, Fig. 3). The median NIHSS score at 3 months was 5.5 points in both

treatment arms, and at month 24, patients treated with AD-MSCs showed nonsignificantly lower median scores (3 [3–6.75] vs 7 [0–12.5]) compared with the placebo group.

An analysis of the serum biomarkers showed higher median basal values of BDNF in the AD-MSC treatment group (710 [589.85–836.4] UI/ml vs 204.6 [193.3–219] UI/ml, $P = 0.014$). No significant differences were detected in BDNF levels at the other measured time points (7 days and 3 months); however, increasing BDNF values from baseline were observed in the placebo group, whereas these values in the AD-MSC group decreased, and this different trend was significant ($P = 0.014$). The same significant trend was observed in VEGF levels, although no differences between treatment groups in any of the measured time points were detected (Fig. 4). Regarding MMP-9 and EVs, there were no differences in their levels between treatment groups at any of the measured time points.

Discussion

According to the results of the AMASCIS trial, intravenous infusion of allogeneic AD-MSCs within the first 2 weeks from ischemic stroke symptom onset is safe and well tolerated at a unique dose of 1 million cells per kilogram. No infusion reactions occurred, and there was no observed association between the reported AEs and AD-MSC

Table 1. Baseline and Demographic Characteristics of the Study Population.

	AD-MSC $n = 9$	Placebo $n = 10$	P value
Sex			
Male	1 (11.1%)	3 (30.0%)	0.582
Female	8 (88.8%)	7 (70.0%)	
Age (years)	78 (70.5–82)	76 (69–80.25)	0.437
Hypertension	6 (66.7%)	9 (90%)	0.303
Diabetes	2 (22.2%)	2 (20%)	1.000
Dyslipidemia	5 (55.6%)	6 (60%)	1.000
Smoker			0.929
Current	1 (11.1%)	1 (10%)	
Former	2 (22.2%)	3 (30%)	
Metabolic syndrome	1 (11.1%)	2 (20%)	1.000
Coronary arterial disease	0 (0%)	1 (10%)	1.000
Atrial fibrillation	5 (55.6%)	8 (80%)	0.350
Previous stroke	2 (2.22%)	2 (20%)	1.000
Weight (kg)	65 (61–73.5)	71 (65–86)	0.164
Modified Rankin Scale (mRS) at baseline			0.582
mRS 0 points	7 (77.8%)	9 (90%)	
mRS 1 point	2 (22.2%)	1 (10%)	
NIHSS baseline (V1, screening)	12 (9–17.5)	10.5 (9.5–14)	0.325
NIHSS pretreatment (V2)	9.5 (6.75–13.75)	9 (6–11.5)	0.697
Capillary glucose levels at baseline	99 (85–144)	124 (98.5–142.5)	0.453
Reperfusion therapy			0.189
None	7 (77.8%)	4 (40%)	
Intravenous thrombolysis (IVT)	1 (11.1%)	1 (10%)	
Mechanical thrombectomy (MT)	0 (0%)	4 (40%)	
Both (IVT and MT)	1 (11.1%)	1 (10%)	
Infarct size (ml)	43.22 (37.57–94.01)	88.165 (55.06–130.75)	0.414
Stroke etiological subtype			0.131
Large vessel disease	1 (11.1%)	0 (0%)	
Cardioembolic	5 (55.5%)	10 (100%)	
Unknown	1 (11.1%)	0 (0%)	
Uncommon etiology	2 (22.2%)	0 (0%)	
Time (days) since stroke symptom onset to study treatment administration	13 (13–13.75)	12 (10–13)	0.028

AD-MSCs: adipose-derived mesenchymal stem cells; NIHSS: National Institute of Health Stroke Scale.

administration. To our knowledge, this is the first clinical trial evaluating treatment with intravenous allogeneic AD-MSCs in patients with ischemic stroke. This type of cell therapy has subsequently been tested in other nonneurologic diseases, such as acute respiratory distress[22] and treatment-refractive rheumatoid arthritis[23], without significant safety issues.

Although the beneficial effect of intravenous AD-MSCs in ischemic stroke has been already demonstrated in animal models, even with stroke-associated comorbidities[16,17,24,25], the AMASCIS trial was underpowered to detect significant differences in secondary outcome efficacy due to its exploratory nature and limited sample size. Although there appeared to be a trend toward lower NIHSS scores in the AD-MSC treatment allocation group, no differences in functional outcome as measured by the mRS were noted. Recently, the positive effect of intravenous cell therapy using allogeneic multipotent adult progenitor cells has been suggested in "Multistem in Acute Stroke Treatment to Enhance Recovery Study (MASTERS)", in which 129 patients were randomized to receive 1,200 million cells between 24 and 48 h from symptom onset[7]. No dose-limiting toxicity, infusional, or allergic reactions were detected during follow-up, and there was no difference in global stroke recovery at day 90 between the treatment and placebo groups. A *post hoc* analysis, however, showed an improvement in the mRS distribution at 90 days in an early window of the treatment administration group (<36 h) and also a higher percentage of excellent outcomes (defined as a composite end point of mRS ≤1, NIHSS ≤1, and Barthel index ≥95). After 1 year of follow-up, the early treatment group showed significant differences in

Table 2. List of SAEs.

SAE	Treatment arm	Time since treatment administration to SAE	Relation between study treatment and SAE	Outcome
Multiorgan failure	Placebo	24 hours	Possibly related	Death
Aspiration pneumonia	Placebo	24 months	Not related	Recovered
Ischemic stroke recurrence	Placebo	18 months	Not related	Recovered with sequelae
Functional decline	Placebo	18 months	Not related	Recovered with sequelae
Neurological decline	Placebo	24 months	Not related	Recovered
Decompensated cardiac insufficiency	Placebo	24 months	Not related	Recovered
Sepsis due to urinary infection	Placebo	24 months	Not related	Recovered
Acute pancreatitis of biliary origin	Placebo	3 months	Not related	Recovered
Recurrent epileptic seizures with cardiac arrest	Placebo	5 months	Not related	Recovered
Hematuria	AD-MSCs	3 months	Not related	Recovered
Hypernatremia	AD-MSCs	3 months	Not related	Recovered

AD-MSCs: adipose-derived mesenchymal stem cells; SAE: serious adverse event.

Table 3. Predefined Systemic and Neurological Complications.

	AD-MSCs n = 4	Placebo n = 9	P value
Deaths	0 (0%)	1 (11.1%)	1.0
No. of patients with SAEs	1 (25%)	4 (44.4%)	1.0
Patients with neurological complications			
Deteriorating stroke	0 (0%)	0 (0%)	
Stroke recurrence	1 (25%)	1 (11.1%)	1.0
Brain edema	0 (0%)	0 (0%)	
Seizures	0 (0%)	2 (22.2%)	1.0
Symptomatic hemorrhagic transformation	0 (0%)	0 (0%)	
Patients with systemic complications			
Respiratory infection	1 (25%)	3 (33.3%)	1.0
Urinary tract infection	3 (75%)	5 (55.6%)	1.0
Deep vein thrombosis	0 (0%)	1 (11.1%)	1.0
Pulmonary embolism	0 (0%)	0 (0%)	
Gastrointestinal hemorrhage	0 (0%)	0 (0%)	
Tumor development	0 (0%)	0 (0%)	

AD-MSCs: adipose-derived mesenchymal stem cells; SAE: serious adverse event.

several clinical outcomes. Compared with the MASTERS trial population, the participants in the AMASCIS trial were older and with a predominance of women. We consider this to prove the safety of MSC therapy in populations often underrepresented in stroke trials, contributing to current knowledge in this field. The "Intravenous stem cells after ischemic stroke-Heuristic value of multimodal MRI to assess MEsenchymal stem cell therapy in Stroke (ISIS-HERMES)" clinical trial[26], in which 16 of 31 patients were treated with autologous bone marrow–derived MSCs in the 2 weeks after stroke onset, showed significant improvements in NIHSS motor scores and Fugl-Meyer motor scores, although no differences were observed with the placebo group regarding mRS, full NIHSS score, or Barthel Index at 6 months and 2

years after treatment administration. Long-term efficacy of MSC therapy in ischemic stroke has also been suggested in an open-label, observer-blinded clinical trial[27] in which 16 patients were treated with autologous bone marrow–derived MSCs (vs 36 patients in the control group) within 7 weeks from stroke onset and follow-up for 5 years. No differences in neurologic complications occurred between the treatment groups, and a significant improvement in mRS scores was reported. Therefore, there appears to be a tendency toward efficacy in the short and long term with MSC therapies; however, the most appropriate cell type, as well as the manner and time of administration is yet to be determined.

Currently, there are various suggested mechanisms to explain the possible therapeutic effect of mesenchymal-derived stem
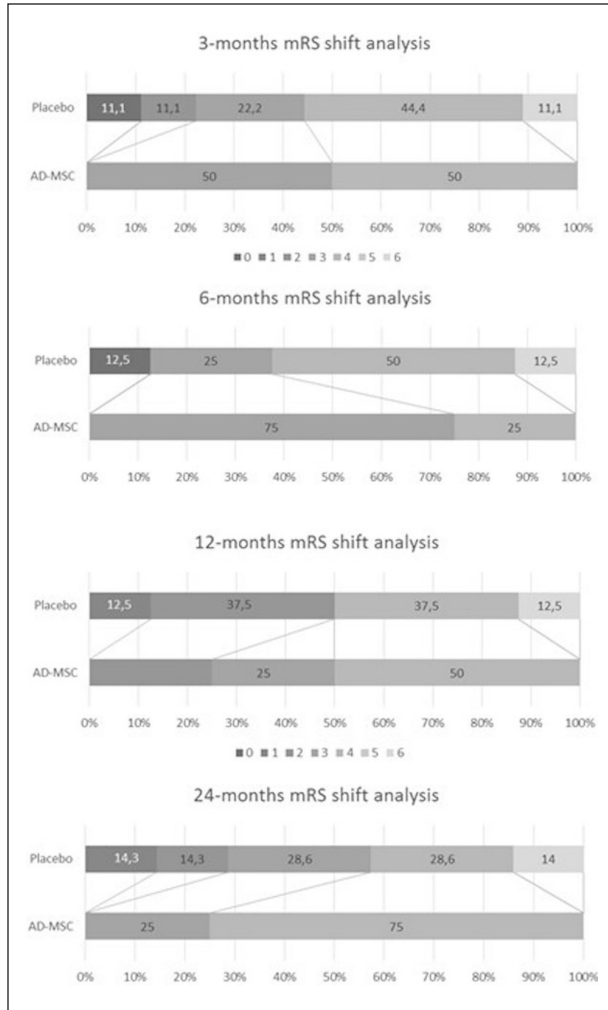
Figure 2. mRS shift analysis. AD-MSCs: adipose-derived mesenchymal stem cells; mRS: modified Rankin Scale.

Table 4. NIHSS Scores.

NIHSS score	AD-MSCs	Placebo	*P* value
Screening (V0)			
Median [IQR]	10.5 [8.25–18]	11 [9–14]	1.000
Max	20	17	
Min	8	8	
Pretreatment (V1)			
Median [IQR]	9.5 [6.75–13.75]	9 [6–11.5]	0.697
Max	15	17	
Min	6	1	
2 h post-treatment (V2)			
Median [IQR]	9.5 [6.75–13.75]	9 [6–11.5]	0.697
Max	15	17	
Min	6	1	
24 h post-treatment (V3)			
Median [IQR]	8 [5.5–13.5]	9 [5–14.5]	0.699
Max	15	17	
Min	5	1	

(continued)

Table 4. (continued)

NIHSS score	AD-MSCs	Placebo	*P* value
7 days (V4)			
Median [IQR]	8.5 [5–12.75]	8 [2.25–10.5]	0.608
Max	14	13	
Min	4	0	
Month 3 (V5)			
Median [IQR]	5.5 [2–6]	5.5 [1.25–6.75]	0.861
Max	6	8	
Min	1	0	
Month 6 (V6)			
Median [IQR]	2.5 [1.25–4.5]	5 [3–8.5–8]	0.199
Max	5	10	
Min	1	0	
Month 12 (V7)			
Median [IQR]	2.5 [1.25–5.25]	3 [0.75–5.75]	1.000
Max	6	8	
Min	1	0	
Month 18 (V8)			
Median [IQR]	2.5 [2–5.25]	7 [1.5–9.5]	0.306
Max	6	10	
Min	2	0	
Month 24 (V9)			
Median [IQR]	3 [3–6.75]	7 [0–12.5]	0.900
Max	8	17	
Min	3	0	

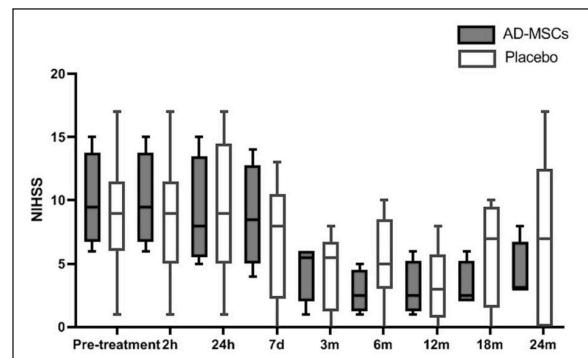AD-MSCs: adipose-derived mesenchymal stem cells; NIHSS: National Institute of Health Stroke Scale.

Figure 3. NIHSS scores up to 24 months of follow-up. AD-MSCs: adipose-derived mesenchymal stem cells; NIHSS: National Institute of Health Stroke Scale.

cells in stroke, such as cell migration, modulation of the immune response, brain protection, and stimulation of endogenous brain repair processes[28]. When MSCs are implanted by intravenous injection, the mechanisms that are probably most relevant in brain repair would be those derived from their paracrine function, involving secretion of cytokines and growth factors that favor reformation of the neurovascular units, endogenous neurogenesis, neuroblast migration, and axonal growth and plasticity. Among various trophic factors implicated in these functions are VEGF and BDNF. In our study, we observed
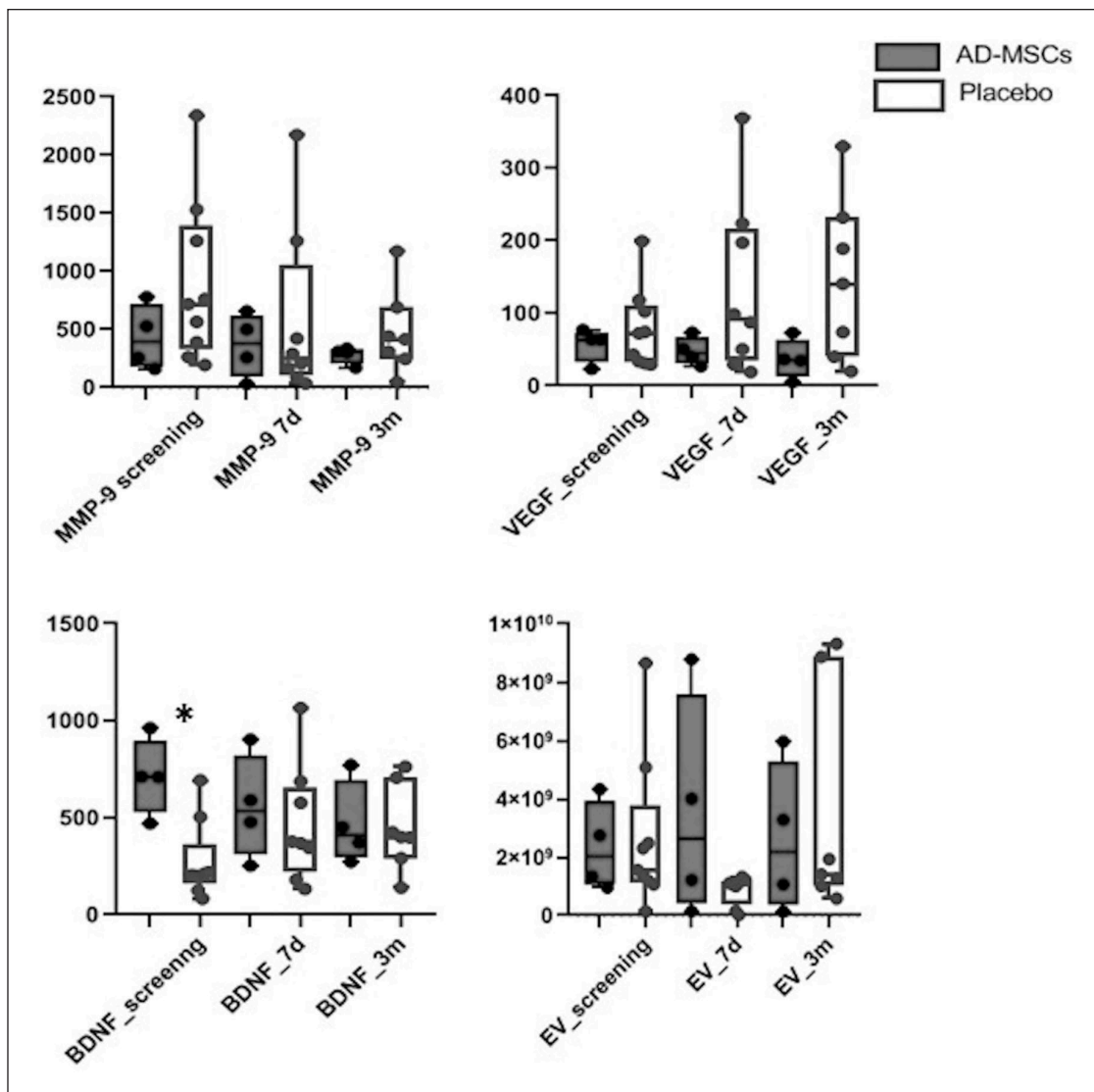
Figure 4. Levels of serum biomarkers. BDNF: brain-derived neurotrophic factor; EV: extracellular vesicle; MMP9: matrix metalloproteinase-9; VEGF: vascular endothelial growth factor.

slightly increasing values of both VEGF and BDNF in the placebo treatment group in contrast to the subtle decrease in values in the AD-MSC group, a fact that appears contradictory. However, taking into account the small sample size and intragroup variability, we consider this observation scarcely reliable. EVs secreted by MSCs are spherical cytoplasmatic components of 50 to 150 nm in size that contain large numbers of bioactive components, such as proteins, lipids, growth factors, cytokines, chemokines, messenger RNAs (mRNAs), and microRNAs that function as messengers between the MSCs and the injured brain tissue cells[29] and contribute to regeneration of the neurovascular unit. In a recent preclinical study in rats with subcortical ischemic stroke[30] performed by our study group, the early intravenous administration of EVs derived from AD-MSCs showed significant functional recovery, a reduction in white matter injury, generation of neurovascular unit cells, and a reduction in

cell death compared with the placebo group, demonstrating their importance in stroke recovery. In the AMASCIS trial, no differences were observed in EV secretion between treatment groups, but again, this result is limited by the sample size.

One of the main remaining questions regarding stem cell therapy is the optimal time window for their administration. Most preclinical studies are performed in acute stages of stroke, but clinical trials are usually conducted at later stages. Taking into account the proposed mechanisms of action of MSCs that imply modulation of inflammation responses of the ischemic tissue, the benefit of stem cell administration would appear greater in the earliest phases of ischemic stroke. The promising efficacy results in the early treatment administration group of the MASTERS trial support this theory.

Therefore, considering the safety results from the AMASCIS study, we are currently recruiting patients for the

AMASCIS-02 trial (NCT04280003), in which an earlier time window will be explored for safety and efficacy results in 30 patients with ischemic stroke[31].

The main limitation of the AMASCIS study is the small sample size due to its pilot clinical trial design and the low number of patients in the treatment group as a consequence of technical issues during the manufacturing process and the development of exclusion criteria after randomization.

In conclusion, the AMASCIS pilot phase IIa clinical trial results suggest that the intravenous administration of AD-MSCs within the first 2 weeks of ischemic stroke onset is safe at 24 months of follow-up. Although no efficacy end points were statistically significant between treatment groups, a trend toward improvement was observed in the NIHSS scores in the AD-MSC treatment arm compared with placebo.

Acknowledgments

Contributors

EdC-R wrote the first draft of the manuscript and was involved in data interpretation and statistical analysis; BF was involved in the writing of the manuscript, study design, data interpretation, and patient enrollment. RG-Z, MAdL, and GR-A enrolled patients. MG-F, LO-O, FL-G, and MCGdF performed the biomarker analysis and interpretation. AB contributed to the statistical analysis. ED-T performed the study design, data interpretation, and writing of the manuscript.

Ethical Approval

Ethical approval to perform this clinical trial was obtained from La Paz University Hospital Ethics Committee (approval code: 3479) and by the Spanish Agency of Medicines and Health Products in 2011 (EudraCT: 2011-003551-18).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the La Paz University Hospital Ethics Committee's approved protocols (approval code: 3479). This article does not contain any studies with animal subjects.

Statement of Informed Consent

Written informed consent was obtained from the patient or their legally authorized representative for anonymized patient information to be published in this article.

Trial Registry Name and Number

Reparative Therapy in Acute Ischemic Stroke With Allogenic Mesenchymal Stem Cells From Adipose Tissue, Safety Assessment, a Randomized, Double Blind Placebo Controlled Single Center Pilot Clinical Trial (AMASCIS-01). NCT01678534. EudraCT: 2011-003551-18

Data Sharing

The data that support the findings of this study are available from the corresponding author on request.

Declaration of Conflicting Interests

Funding

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Supplemental Material

Supplemental material for this article is available online.

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