Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background Multipotent adult progenitor cells are a bone marrow-derived, allogeneic, cell therapy product that modulates the immune system, and represents a promising therapy for acute stroke. We aimed to identify the highest, well-tolerated, and safest single dose of multipotent adult progenitor cells, and if they were efficacious as a treatment for stroke recovery.

Methods We did a phase 2, randomised, double-blind, placebo-controlled, dose-escalation trial of intravenous multipotent adult progenitor cells in 33 centres in the UK and the USA. We used a computer-generated randomisation sequence and interactive voice and web response system to assign patients aged 18–83 years with moderately severe acute ischaemic stroke and a National Institutes of Health Stroke Scale (NIHSS) score of 8–20 to treatment with intravenous multipotent adult progenitor cells (400 million or 1200 million cells) or placebo between 24 h and 48 h after symptom onset. Patients were ineligible if there was a change in NIHSS of four or more points during at least a 6 h period between screening and randomisation, had brainstem or lacunar infarct, a substantial comorbid disease, an inability to undergo an MRI scan, or had a history of splenectomy. In group 1, patients were enrolled and randomly assigned in a 3:1 ratio to receive 400 million cells or placebo and assessed for safety through 7 days. In group 2, patients were randomly assigned in a 3:1 ratio to receive 1200 million cells or placebo and assessed for safety through the first 7 days. In group 3, patients were enrolled, randomly assigned, and stratified by baseline NIHSS score to receive 1200 million cells or placebo in a 1:1 ratio within 24–48 h. Patients, investigators, and clinicians were masked to treatment assignment. The primary safety outcome was dose-limiting toxicity effects. The primary efficacy endpoint was global stroke recovery, which combines dichotomised results from the modified Rankin scale, change in NIHSS score from baseline, and Barthel index at day 90. Analysis was by intention to treat (ITT) including all patients in groups 2 and 3 who received the investigational agent or placebo. This study is registered with ClinicalTrials.gov, number NCT01436487.

Findings The study was done between Oct 24, 2011, and Dec 7, 2015. After safety assessments in eight patients in group 1, 129 patients were randomly assigned (67 to receive multipotent adult progenitor cells and 62 to receive placebo) in groups 2 and 3 (1200 million cells). The ITT populations consisted of 65 patients who received multipotent adult progenitor cells and 61 patients who received placebo. There were no dose-limiting toxicity events in either group. There were no infusional or allergic reactions and no difference in treatment-emergent adverse events between the groups (64 [99%] of 65 patients in the multipotent adult progenitor cell group *vs* 59 [97%] of 61 in the placebo group). There was no difference between the multipotent adult progenitor cell group and placebo groups in global stroke recovery at day 90 (odds ratio 1·08 [95% CI 0·55–2·09], p=0·83).

Interpretation Administration of multipotent adult progenitor cells was safe and well tolerated in patients with acute ischaemic stroke. Although no significant improvement was observed at 90 days in neurological outcomes with multipotent adult progenitor cells treatment, further clinical trials evaluating the efficacy of the intervention in an earlier time window after stroke (<36 h) are planned.

Funding Athersys Inc.

Introduction

Intravenous tissue plasminogen activator (tPA; alteplase) and endovascular thrombectomy are effective treatments for stroke. However, the time window for these treatments is limited to 4·5 h for tPA and 6·0 h in the UK and USA for endovascular thrombectomy from symptom onset, and endovascular thrombectomy requires specialised stroke expertise and endovascular skills available mainly at comprehensive stroke centres.[1,2] Less than 5% of patients with ischaemic stroke benefit from these therapies and, even with endovascular thrombectomy, up to 50% of patients cab due or be disabled at 90 days.[2] Hence, there is a large unmet need for safe, effective, and widely available treatments for acute stroke beyond 6 h from symptom onset.

Cell therapy for stroke has been shown in animal models to be a promising strategy to limit ischaemic injury and promote recovery after ischaemic stroke in

Research in context

Evidence before this study

We searched PubMed up to Oct 24, 2016 using the search terms "cell therapy AND stroke", 'mesenchymal stem cells AND stroke", and "bone marrow-derived stem cells AND stroke"; the search was restricted to English language papers. There have been less than ten cell therapy trials of intracerebral injection of neural or neutralised stem cells in stroke, less than ten single centre trials of intravenous autologous bone marrow-derived cells and mesenchymal stem cells, and less than ten small intra-arterial cell therapy trials in stroke. Many of these trials have shown safety and some promise of efficacy in stroke treatment. However, there have been no large multicentre, randomised, placebo-controlled trials of an allogeneic bone marrow-derived cell therapy.

Added value of this study

This randomised, double-blind, phase 2, placebo-controlled trial of an allogeneic cell therapy with no required tissue matching showed feasibility of a multicentre cell-therapy trial in stroke, and the safety and tolerability of multipotent adult progenitor cells treatment. Although the primary efficacy outcome of multivariate global stroke recovery and secondary outcomes showed no difference between groups, post-hoc analyses of patients treated earlier in the time window between 24 h and 36 h suggest benefit in outcome at 1 year follow-up that requires confirmation in future trials.

Implications of all available evidence

Stroke is the leading cause of disability worldwide in adults, yet treatment for stroke is a huge unmet need. Cell therapy is a promising treatment avenue for stroke therapy. This trial indicates that multipotent adult progenitor cells therapy is safe, tolerable, and feasible in multicentre clinical trials. Treatment efficacy of multipotent adult progenitor cells needs to be explored in future trials within the 18–36 h time window after stroke onset.

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extended time windows.[3,4] Cell therapy approaches include different cell types (eg, mesenchymal stem cells, bone marrow mononuclear cells, and neural stem cells), routes of administration (eg, intravenous, intra-arterial, or intracerebral), and time windows (days to months).[3,5] In two small phase 1, single arm, open-label clinical trials in patients with stroke,[6,7] intracerebral delivery of either a neural stem cell line or a mesenchymal stem cell line were shown to be safe. In the first week after stroke, the immune system is activated with splenocytes and other immune cells targeting the ischaemic brain, possibly aggravating ischaemic damage, and preventing remodelling and recovery.[4,8] This period is probably an optimum time window for intravenous administration of bone marrow-derived cell therapies to provide therapeutic benefit given their immunomodulatory effects.[3] Intravenous administration of autologous bone marrow-derived cells is safe, but requires expansion time in culture that prohibits administration of a therapeutically relevant number of cells to the patient in the first week.[9,10] A more optimised approach is an allogeneic cell therapy product administered intravenously, that is scalable and universal, and requires no tissue matching. Multipotent adult progenitor cells are a plastic, adherent, bone marrow derived population of adult progenitor cells first characterised more than a decade ago.[11,12] Clinical grade multipotent adult progenitor cells are isolated from a healthy unrelated donor and are an allogenic universal cell therapy with long-term culture expansion and potency.[13,14] Compared with other adherent cells, such as mesenchymal stem cells, multipotent adult progenitor cells have an extended differentiation capability,[15] and distinct phenotype and functional characteristics,[15] transcriptome,[15] secretome,[16] miRNA profiles, and size.[17]

We did a phase 2, multicentre, double-blind, randomised, parallel group, placebo-controlled trial of multipotent adult progenitor cells treatment in patients with moderately severe acute ischaemic stroke.[18] In the MultiStem in Acute Stroke Treatment to Enhance Recovery Study (MASTERS), we aimed to establish the highest, well-tolerated, and safest single dose of multipotent adult progenitor cells up to a maximum of 1200 million total cells, and if there was efficacy as a treatment for stroke recovery.

Methods

Study design

We did a phase 2 multicentre, randomised, double-blind, placebo-controlled, dose-escalation trial of intravenous multipotent adult progenitor cells compared with placebo.[18] We enrolled and randomly assigned patients in two escalating dose tiers (groups 1 and 2) and then chose the highest well tolerated dose for administration to patients in group 3. The study was done in 33 medical centres in the UK and in the USA, and was approved by local institutional review boards and ethics committees.

Patients

Patient enrolment began on Oct 24, 2011, and the last patient follow-up visit was on Dec 7, 2015. We initially enrolled patients aged 18–79 years with a moderately severe ischaemic stroke with motor or speech deficit defined by a National Institutes of Health Stroke Scale (NIHSS) score of 8–20 at baseline just before administration (≥24 h). To be enrolled, patients needed to have confirmation of a hemispheric cortical infarct in the anterior circulation with brain MRI including diffusion-weighted imaging showing an acute lesion measuring more than 5 mL and less than 100 mL. According to the

trial protocol, patients who either received tPA or endovascular thrombectomy (but not patients receiving both) were to be included in the study. Initially, multipotent adult progenitor cells or placebo had to be administered between 24 h and 36 h after onset of stroke symptoms, but the treatment window was extended to 48 h during the course of the study.

Patients were ineligible if there was a change in NIHSS score of four or more points during at least a 6 h period between screening and randomisation. We excluded patients with brainstem or lacunar infarct; a substantial comorbid disease such as severe congestive heart failure, chronic obstructive pulmonary disease, or renal or hepatic failure; an inability to undergo an MRI scan, and with a history of splenectomy. A complete list of inclusion and exclusion criteria is provided in the appendix.

See Online for appendix

In response to lower than expected enrolment rates in the early stages of the study, the protocol's inclusion and exclusion criteria were amended after about 30 patients were randomly assigned to broaden the eligible patient population (amended July 26, 2013, and approved by all local ethic committees). First, the upper age limit was increased from 79 years to 83 years. Second, we expanded the treatment window from 24–36 h to 24–48 h. We updated this treatment window to address an important logistical issue at many clinical centres, namely, the limited hours of operations of cell processing laboratories for thawing, dose configuration, and preparation of cell material, which were required for the first generation of multipotent adult progenitor cells product configuration used in this study. Finally, because of the increasing use of endovascular thrombectomy at many of the centres, we allowed for the inclusion of patients receiving both tPA treatment and endovascular thrombectomy. All patients provided written informed consent for participation.

Randomisation and masking

Through a computer generated process and interactive voice and web response system (Medpace, Cincinnati, OH, USA), we randomly assigned patients in group 1 in a 3:1 ratio to receive an intravenous infusion of 400 million multipotent adult progenitor cells or placebo within 24–36 h of stroke onset. After review by an independent safety committee, we randomly assigned additional patients in group 2 in a 3:1 ratio to receive an intravenous infusion of 1200 million multipotent adult progenitor cells or placebo within 24–36 h of stroke onset. In group 3, additional patients were randomly assigned in a 1:1 ratio to receive an intravenous infusion dose of 1200 million multipotent adult progenitor cells or placebo within 24–48 h of stroke onset. Randomisation in group 3 was stratified by baseline NIHSS score (≤12 and ≥13).

At each site, a designated staff member in the cell processing facility who was unblinded to patient treatment assignments contacted the interactive voice or web response system to acquire the treatment assignment, and then prepared and dispensed the investigational product. This staff member had no further involvement with the patient for the rest of the trial. Treatment assignments for the individual patients were assigned through a computer generated randomisation list. Patients and all trial personnel, including investigators, and clinicians, were blinded to treatment assignment. A tinted cover and sleeve were applied to the intravenous infusion bag and tubing.

Procedures

MultiStem multipotent adult progenitor cells (Lonza, Walkersville, MD, USA; under contract with Athersys, Inc) were provided to the clinical sites in standard units frozen in PlasmaLyte-A, dimethyl sulfoxide, and human serum albumin, and were thawed, combined, and formulated to the appropriate dose of either 400 million or 1200 million cells. The matching placebo contained PlasmaLyte-A, dimethyl sulfoxide, and human serum albumin in the same concentrations. We administered multipotent adult progenitor cells or placebo intravenously by gravity for about 1 h once between 24 h and 48 h after stroke onset.

Patients were assessed by study personnel at day 7, day 30, day 90, and day 365 after receiving the investigational product with the modified Rankin scale (mRS), NIHSS, and Barthel index scales at scheduled visits. Additionally, patients were contacted by telephone at 60 days, and every month after 90 days to update their medical status. An MRI brain scan was done at baseline, and day 30, and day 365 after treatment, and blood for inflammatory biomarkers was taken at baseline, and day 2, day 7, and day 30 after treatment.

To measure biomarkers in serum, multiplex immunoassays were done by Aeirtec (Newcastle upon Tyne, UK). At baseline, day 2, day 7, and day 30 after treatment, we measured: interleukin 1 β, interleukin 2, interleukin 6, interleukin 10, interleukin 12, interferon-γ, monocyte chemotactic protein 1, and tumour necrosis factor α. CD3 positive lymphocytes and FoxP3 positive regulatory T cells were measured in the blood at baseline, days 2, 7, and 30 by an epigenetic assay by Epiontis (Berlin, Germany; appendix).[19]

Outcomes

The primary efficacy outcome was the multivariate global stroke recovery at day 90, which assesses global disability, neurological deficit, and activities of daily living and consists of mRS 2 or less; NIHSS total score improvement of 75% or more from baseline; and Barthel index of 95 or more in the multipotent adult progenitor cells treatment group, compared with the placebo treatment.

The secondary efficacy outcomes were the functional outcome throughout the range of modified Rankin scores measured by shift analysis at day 90; the proportion of patients with an mRS score of 2 or less

(scale 0–6) at day 90; the proportion of patients with a total NIHSS score improvement of more than 75% from baseline at day 90; the proportion of patients with a Barthel index score of 95 or more (scale 0–100) at day 90; the proportion of patients with a total NIHSS score of less than 1, or by more than 11 point improvement from baseline at day 90; and the proportion of patients with an excellent outcome at day 90 defined by all of the following criteria: mRS score 0–1 (scale 0–6); NIHSS total score 0–1; and Barthel index score ≥95 (scale 0–100).

The exploratory efficacy endpoints were the difference between multipotent adult progenitor cells and placebo in the following: changes in cortical infarct volume as measured by MRI from baseline to day 30 and day 365; changes in blood biomarkers (white blood cells and inflammatory biomarkers) from baseline to day 2, day 7, and day 30; changes in mRS, NIHSS, and Barthel index from baseline to day 7, day 40, day 90, and day 365; the proportion of patients with good outcomes or substantial improvement (ie, mRS of ≤2, mRS improvement of ≥2 points, and NIHSS total score improvement of ≥75%) at day 7, day 30, or day 365; global stroke recovery at day 90 with overall disease improvement across the three binary variables (mRS ≤1, NIHSS ≤1, and a Barthel index score of ≥95); and functional outcome throughout the range of mRS by shift analysis at day 7 and day 30 separately.

The primary safety endpoint was dose-limiting toxic events at 7 days after infusion, defined as any of the following: Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or 4 infusion-related allergic adverse events that were related to the investigational product occurring in the first 24 h after infusion; CTCAE grade 3 or 4 adverse events that were related to investigational product assessed at 7 days after infusion; or neurological worsening that was related to investigational product and defined as a four point or more increase in NIHSS compared with baseline NIHSS assessed through day 7 after infusion. Secondary safety outcomes included the incidence of secondary infections (local and systemic) and the differences in other safety assessments including adverse events, mortality, vital signs (ie, blood pressure, heart rate, respiration rate, temperature, and oxygen saturation), and laboratory parameters through day 365.

Statistical analysis

Patients enrolled in groups 2 and 3 who were randomly assigned to receive the highest dose of multipotent adult progenitor cells (1200 million cells) or placebo equivalent, constituted the assessable population. The primary, secondary, and exploratory efficacy outcomes (except for blood biomarkers) in groups 2 and 3 were analysed in the intention-to-treat (ITT) population, which comprised all patients who were randomly assigned to receive 1200 million cells or placebo equivalent. Safety outcomes were also assessed in the ITT population for groups 2 and 3. Blood biomarkers were analysed in the modified ITT population, which included all patients who were randomly assigned to receive multipotent adult progenitor cells treatment and had at least one post-infusion efficacy assessment at day 7 or later.

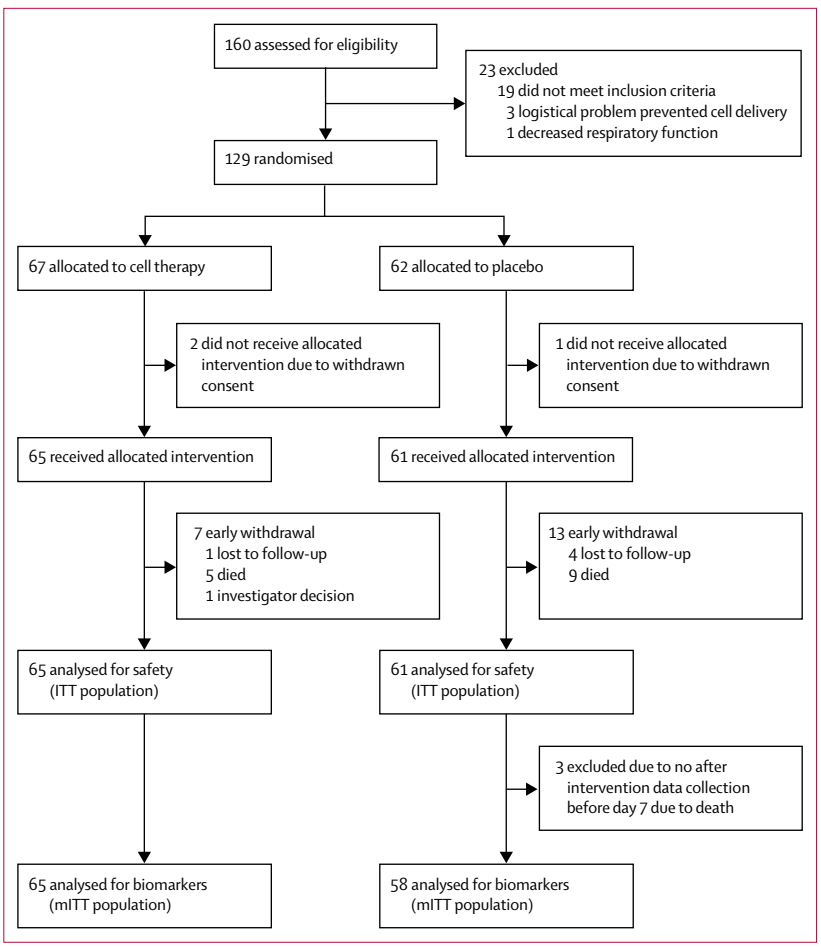

Figure: **Trial profile for groups 2 and 3**
ITT=intention-to-treat. mITT=modified intention-to-treat. In group 1, out of nine patients screened for eligibility, eight patients were recruited. Six patients were allocated to receive cell therapy and two patients to receive placebo.

The primary efficacy endpoint assessed the global stroke recovery by use of mRS, NIHSS, and the Barthel index. The data from these three binary variables from each patient were analysed with an additive logistic regression model with the treatment group and baseline NIHSS score (≤12 or ≥13) as dependent variables. The standard generalised estimating equations technique, which handles correlated outcomes, was used to make inferences about the treatment difference by testing the null hypothesis that the odds ratio of a favourable outcome based on the three outcome measures was equal in the two groups. An exchangeable correlation structure was used to model the correlations among response variables. SAS PROC GENMOD (version 9.3) was used to perform the analysis. The standard estimation method for generalised estimating equations for the treatment difference (average for the three

outcome variables) was also obtained, including a 95% CI. Categorical data were summarised with absolute frequencies or relative percentages and continuous data were summarised with means and standard deviations. For secondary outcomes, the last observation carried forward principle applied for early termination patients or missing values through day 90, and the binary outcomes are reported with two-sided p values from the Cochran-Mantel-Haenszel test controlling for baseline severity by NIHSS category (≤12 or ≥13).

Using simulations designed to assess the power of global stroke recovery, we calculated that a sample of approximately 125 patients (65 in the treatment group and 60 in the control group) would yield a power of more than 90%, at a significance level of 0·05, to detect a treatment effect between the treatment and control groups, based on treatment differences in the binary subcomponents of 10% in mRS response, 20% in NIHSS response, and 20% in the Barthel Index response.

Patients with combined intravenous tPA and endovascular thrombectomy might have rapidly improved before the screening examination and were likely to achieve a good outcome regardless of group assignment, confounding the results. To explore the effects of key protocol changes on the study's results, we did a post-hoc analysis of patients treated within 36 h, excluding those treated with combined intravenous tPA and endovascular thrombectomy.

	Group 1		Groups 2 and 3 combined	
	Multipotent adult progenitor cells (n=6)	Placebo (n=2)	Multipotent adult progenitor cells (n=65)	Placebo (n=61)
Age	55·0 (9·7)	59·0 (21·2)	61·8 (11·4)	62·6 (11·4)
≥65 years	0 (0%)	1 (50%)	28 (43%)	28 (46%)
Sex				
Male	5 (83%)	1 (50%)	35 (54%)	33 (54%)
Female	1 (17%)	1 (50%)	30 (46%)	28 (46%)
Patients with left hemisphere event	5 (83%)	2 (100%)	37 (57%)	36 (59%)
Infarct size (mL)	55·8 (27·1)	9·3 (1·1)	43·7 (26·9)	50·9 (41·3)
Patients who had reperfusion therapy (tPA, endovascular thrombectomy, or both)	0 (0%)	1 (50%)	38 (59%)	32 (53%)
tPA	0 (0%)	1 (50%)	29 (45%)	29 (48%)
Endovascular thrombectomy	0 (0%)	0 (0%)	17 (26%)	12 (20%)
Both tPA and endovascular thrombectomy	0 (0%)	0 (0%)	8 (12%)	9 (15%)
Mean NIHSS at baseline	12·2 (2·9)·	15·5 (5·0)	13·4 (3·6)	13·3 (3·7)
Median NIHSS at baseline	12 (9–17)	13 (9–19)	13 (8–20)	13 (8–20)
NIHSS 8–12 at baseline	3 (50%)	1 (50%)	29 (45%)	27 (44%)
Symptom onset to drug infusion (h)	31·7 (2·8)	32·8 (3·4)	37·2 (6·9)	39·3 (6·7)

Data are mean (SD), n (%), or median (range). NIHSS=National Institutes of Health Stroke Scale. tPA=tissue plasminogen activator.

Table 1: **Baseline characteristics**

This study is registered with ClinicalTrials.gov, number NCT01436487.

Role of the funding source

The funder of the study was involved in study design and in data interpretation. All data collection and analysis were overseen by Medpace. One employee of the funder (RWM) was represented on the writing committee. The corresponding author 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

This study was done between Oct 24, 2011, and Dec 7, 2015. Of the nine patients screened for group 1, we randomised eight (six received 400 million cells and two received placebo). One patient did not meet the inclusion criteria and was not randomly allocated to a treatment. After this dose was determined to have no safety concerns, we randomly assigned 129 patients for groups 2 and then group 3 (1200 million cells; figure). Of these, 65 patients in the multipotent adult progenitor cells group and 61 patients in the placebo group received the intervention; three patients (two in the multipotent adult progenitor cells group and one in the placebo group) withdrew consent and did not receive the intervention. Demographic and clinical information for all patients in groups 2 and 3 who received multipotent adult progenitor cells or placebo is summarised in table 1. The groups were well matched for age, median NIHSS score, and intravenous tPA treatment. Combined intravenous tPA and endovascular thrombectomy was more frequent in the placebo group (eight [12%] of 65 *vs* nine [15%] of 61). Mean baseline infarct size was larger in the placebo group (table 1).

For the primary efficacy outcome assessed for patients in groups 2 and 3, there was no difference between the multipotent adult progenitor cells and placebo arms in global stroke recovery at day 90 (odds ratio [OR] 1·08 [95% CI 0·55–2·09], p=0·83) and at 1 year (1·48 [0·77–2·84], p=0·24). Table 2 summarises the results of prespecified secondary outcomes. There were no differences between the arms on any of the secondary efficacy outcomes.

Although the primary outcome was evaluated at 90 days, patients were followed up over 1 year for secondary outcomes and 102 (81%) of 126 patients completed the 1 year follow-up. The post-hoc analysis of the 1 year results suggest that patients treated with multipotent adult progenitor cells might have continued to improve over 1 year compared with controls, based on the results from patients completing the 1 year follow-up and patients who withdrew from the study early, with the last observation carried forward and death considered a non-response for dichotomous outcomes. Excellent outcome was different between arms (15 [23%] of 65 in the cell therapy arm *vs* five [8%] of 61 in the

placebo arm; OR 3·59 [95% CI 1·17–10·98]; p=0·021) as was mRS 1 or less (28% *vs* 13%, OR 2·65 [95% CI 1·03–6·80], p=0·041). The Barthel index of 95 or more, distribution of mRS scores (shift), and global stroke recovery were not significantly different between the two arms (table 2).

Multipotent adult progenitor cells therapy was well tolerated throughout the duration of the study. No primary safety endpoint of dose-limiting toxicity events occurred in either group. Life-threatening adverse events, death, and secondary infections were not significantly different between arms (table 2). There were no infusion related allergic reactions and no neurological worsening in either group. There were no clinically meaningful laboratory and electrocardiogram differences between the groups, and no clinically significant vital sign findings after treatment. Treatment-emergent adverse events were not different between the multipotent adult progenitor cells (64 [99%]) and placebo arms (59 [97%]; table 3). Treatment-emergent adverse events related to the investigational product were more frequent in patients treated with multipotent adult progenitor cells (15[23%]) than in those in the placebo group (5[8%]; p=0·018), although most events related to the investigational product were considered mild to moderate. The most common treatment related emergent adverse events were halitosis (six patients in the multipotent adult progenitor cell group *vs* four patients in the placebo group), fever and chills (four *vs* none), and nausea and vomiting (two *vs* none). Overall, there was also no difference in serious adverse events between the arms. Mortality was not different between the arms (five [8%] patients died in the multipotent adult progenitor cell group *vs* nine [15%] patients died in the placebo group; p=0·21).

Average percentages of circulating CD3 positive T cells were reduced at day 2 in patients treated with multipotent adult progenitor cells and increased in patients who received placebo in the modified intent-to-treat population (p=0·001; appendix). There was a difference in FoxP3 positive cells in the blood of the multipotent adult progenitor cells treatment arm *vs* placebo at day 2 after treatment (p=0·010; appendix). There was no difference in concentration of circulating CD3 positive T cells or FoxP3 positive cells between the multipotent adult progenitor cells and placebo arms at days 7 and 30. Inflammatory cytokine levels were also reduced in the multipotent adult progenitor cell arm compared with the placebo arm with differences in tumour necrosis factor α, interleukin 6, and interleukin 1 β at day 7, controlling for differences in baseline levels and missing values in the modified ITT population data (appendix). However, we noted no differences in interleukin 10 between the multipotent adult progenitor cell arms and placebo arms over time.

Table 4 summarises the post-hoc analyses comparing patients receiving multipotent adult progenitor cell administration within 36 h of stroke onset (n=31) with placebo patients (n=61). There was no difference in global stroke recovery at 90 days between arms (OR 1·64 [95% CI 0·75–3·60], p=0·21) or at year 1 (1·62 [0·75–3·49], p=0·22). There was a reduction in secondary infections, but no significant difference in the proportion of patients with life-threatening adverse events or who died in the multipotent adult progenitor cell treatment arm.

	Day 90			1 year*		
	Multipotent adult progenitor cells (n=65)	Placebo (n=61)	p value	Multipotent adult progenitor cells (n=65)	Placebo (n=61)	p value
Efficacy						
mRS ≤2 (scale 0–6)	24 (37%)	22 (36%)	0·93	33 (51%)	27 (44%)	0·46
NIHSS improvement of ≥75%	26 (40%)	23 (38%)	0·79	32 (49%)	28 (46%)	0·71
Barthel index ≥95 (scale 0–100)	30 (46%)	27 (44%)	0·83	40 (62%)	27 (44%)	0·05
NIHSS ≤1 or ≥11 point improvement	25 (39%)	18 (30%)	0·29	..	..	..
mRS shift	..	..	0·29	..	..	0·09
mRS ≤1	10 (15%)	7 (12%)	0·51	18 (28%)	8 (13%)	0·0410
NIHSS ≤1	17 (26%)	10 (16%)	0·17	19 (29%)	12 (20%)	0·20
Excellent outcome†	10 (15%)	4 (7%)	0·10	15 (23%)	5 (8%)	0·0206
Safety						
Life-threatening adverse events or death	..	..	..	8 (12%)	15 (25%)	0·08
Secondary infections	..	..	..	25 (39%)	29 (48%)	0·30
Initial days in hospital	7·6 (4·0)	9·6 (8·1)	0·09	..	..	..

Data are n (%) or mean (SD). Each endpoint was tested independently; no adjustments were made for multiplicity. mRS=modified Rankin Score. NIHSS=National Institutes of Health Stroke Scale. *Assessment of primary and secondary outcomes at 1 year was exploratory.†Excellent outcome is a composite of mRS ≤1, NIHSS ≤1, and Barthel index ≥95.

***Table 2*: Secondary outcomes for groups 2 and 3 combined**

	Multipotent adult progenitor cells (n=65)	Placebo (n=61)
Treatment-emergent adverse event	64 (99%)	59 (97%)
Study drug-related treatment-emergent adverse event*	15 (23%)	5 (8%)
Infusion-related allergic reaction	0 (0%)	0 (0%)
Neurological worsening	0 (0%)	0 (0%)
Secondary infection	25 (39%)	29 (48%)
Serious adverse events	22 (34%)	24 (39%)
Maximum severity of treatment-emergent adverse events		
Mild	12 (18%)	14 (23%)
Moderate	33 (51%)	24 (39%)
Severe	11 (17%)	6 (10%)
Life-threatening	3 (5%)	6 (10%)
Death	5 (8%)	9 (15%)

Data are number of events (%). An adverse event was considered treatment-emergent if the start time of the event was on or after the start of treatment infusion. *An adverse event that was definitely, probably, or possibly related to treatment.

***Table 3*: Treatment-emergent adverse events for groups 2 and 3 combined**

	Day 90			1 year		
	Multipotent adult progenitor cell (n=31)	Placebo (n=61)	p value	Multipotent adult progenitor cell (n=31)	Placebo (n=61)	p value
Efficacy						
mRS ≤2 (scale, 0–6)	14 (45%)	22 (36%)	0·38	16 (52%)	27 (44%)	0·50
Improvement in NIHSS of ≥75%	15 (48%)	23 (38%)	0·33	16 (52%)	28 (46%)	0·61
Barthel index ≥95 (scale 1–100)	18 (58%)	27 (44%)	0·18	22 (71%)	29 (48%)	0·0252
NIHSS ≤1 or ≥11 point improvement	14 (45%)	18 (30%)	0·14	..	..	..
mRS shift	..	..	0·13	..	..	0·07
mRS ≤1 (scale 0–6)	5 (16%)	7 (12%)	0·53	10 (32%)	8 (13%)	0·0281
NIHSS ≤1	10 (32%)	10 (16%)	0·08	11 (36%)	12 (20%)	0·09
Excellent outcome*	5 (16%)	4 (7%)	0·14	9 (29%)	5 (8%)	0·0081
Safety						
Life-threatening adverse events or death	..	..	..	3 (10%)	15 (25%)	0·09
Secondary infections	..	..	..	5 (16%)	29 (48%)	0·0033
Initial days in hospital	6·8 (2·8)	9·6 (8·1)	0·0164	..	..	..

Data are n (%) or mean (SD). Each endpoint was tested independently; no adjustments were made for multiplicity. mRS=modified Rankin Score. NIHSS=National Institutes of Health Stroke Scale. *Excellent outcome is a composite of mRS ≤1, NIHSS ≤1, and Barthel index ≥95.

Table 4: **Post-hoc outcomes for early treatment (<36 h) for groups 2 and 3 combined**

Post-hoc analysis of patients who were treated early (<36 h) and received multipotent adult progenitor cells compared with all patients who received placebo, excluding those with combined intravenous tPA and endovascular thrombectomy (27 patients in the multipotent adult progenitor cell arm *vs* 52 in the placebo arm) did not have greater global stroke recovery at 90 days (appendix; OR 2·28 [95% CI 0·98–5·30], p=0·06). The distribution analysis of mRS scores (shift) was improved in the early treated, multipotent adult progenitor cells arm compared with those in placebo (p=0·028), and excellent outcome was greater in the multipotent adult progenitor cells arm (p=0·034). All other secondary outcomes were non-significant in these post-hoc analyses (appendix). At 1 year, the clinical outcomes improved relative to placebo, with the mRS shift, excellent outcome, and a few other clinical outcomes (the components of excellent outcome: mRS≤1, NIHSS ≤1, and Barthel index ≥95) having significant differences in favour of those patients who received multipotent adult progenitor cells (appendix).

Discussion

Multipotent adult progenitor cell therapy was safe and well tolerated up to a dose of 1200 million cells after a one time intravenous infusion in patients with moderate to moderately severe acute ischaemic stroke. There were no infusion or allergic reactions, no dose limiting toxic events, and adverse events were similar between arms. There was no difference in mortality between arms. In the ITT analysis, there was no difference between the multipotent adult progenitor cells and the placebo arms in the primary efficacy outcome or secondary efficacy outcomes. Although no improvement in efficacy was noted on the primary and secondary efficacy analyses, exploratory analyses suggested an increase in excellent outcome in the multipotent adult progenitor cells arms in the ITT population, and a beneficial clinical effect on long-term 1 year disability. The reason for this finding is unclear but might relate to the cell therapy reducing the secondary neuroinflammatory response and reducing later immunodepression with secondary infections, which might create a better environment for brain recovery.

Time of intervention is a crucial factor in acute stroke trials. The trials of two successful interventions in acute stroke, tPA and endovascular thrombectomy, met with failures related to too late reperfusion. Early trials of tPA were not positive when they enrolled patients later in the 3–6 h time window until the target population was refined to an earlier time window.[20–23] Similarly, early trials of endovascular thrombectomy were not successful due to delayed recanalisation and reperfusion of the brain.[24,25] The targets of multipotent adult progenitor cells for acute stroke are probably the immune system and peripheral immune organs such as the spleen, and cell mediated benefit on these targets is probably time-dependent as well. Splenic activation and inflammation after stroke occur early in rodent models of stroke, within 6–24 h,[26] highlighting the need to target these processes early. Recent studies suggest that there is a time-dependent splenic contraction in patients with acute stroke beginning within 6 h of symptom onset.[27,28] We did various post-hoc analyses to better understand the efficacy and safety of multipotent adult progenitor cells treatment for stroke as a function of time. The rationale for the post-hoc analyses in this trial was based on the initial design and inclusion and exclusion criteria, which targeted a time window of intervention of 24–36 h. This time window was based on animal models in which multipotent adult progenitor cells therapy was most effective if delivered at 24 h after stroke,[29] but was increased during the trial due to feasibility issues of multipotent adult progenitor cells availability at the cell processing facilities. There was no difference in primary outcome efficacy between the arms in the 24–36 h window, although some secondary outcomes were significant in these analyses after we excluded patients who received both tPA and endovascular thrombectomy, which was an initial exclusion criteria.

There are multiple potential mechanisms by which cell therapy might improve outcome after stroke. When bone marrow-derived cells such as multipotent adult progenitor cells are administered intravenously, direct entry and engraftment in the brain is limited and replacement of neurons is unlikely. Evidence suggests that modulation of the immune response might be the primary mode of action of multipotent adult progenitor cells in animal models of acute neurological injury.[3,30,31] The immune response after stroke has both deleterious

and protective functions.[32,33] Stroke is associated with early immune activation and later peripheral immunodepression related to splenic atrophy and so-called immune exhaustion.[8,33] These secondary neuroinflammatory responses contribute to infarct growth and later infections and are more important in strokes affecting larger portions of the brain.[32] Lymphocytes, especially T lymphocytes, appear to be the key leukocyte population in the mediation of the neuroinflammatory response.[32] Multipotent adult progenitor cells at the early timepoint of 24–36 h probably have a neuroprotective effect and preserve neurons from cell death related to this neuroinflammatory response.

We present some of the first data to show that a cell therapy modulates immune responses after acute stroke in human beings. Multipotent adult progenitor cells reduced the secondary peripheral immune responses measured by serum cytokines, CD3 positive T cells, and FoxP3 positive cells, known as regulatory T cells. Regulatory T cells have been identified as having both adverse and beneficial physiological effects in animal models of ischaemic stroke.[32,33] Multipotent adult progenitor cells reduce inflammatory cytokines in the spleen in animal models of acute CNS injury.[30,31] We noted reductions in serum pro-inflammatory cytokines in this trial consistent with an effect on the secondary neuroinflammatory response. We do not know the relationship between blood and tissue cytokines but speculate that there might be similar tissue reductions of inflammatory cytokines. We do not know whether there was an attenuation of reduction in spleen size by multipotent adult progenitor cells in our patients. Measurement of spleen size over time is difficult in patients with acute stroke but is an area of future investigation. The reduction in infections with multipotent adult progenitor cells is also in keeping with its known immunomodulatory effects. Other potential mechanisms of action of multipotent adult progenitor cells are paracrine effects or bystander effects on neurons or promotion of angiogenesis in the brain, but these mechanisms seem less likely with intravenous administration and scarcity of CNS engraftment.

To our knowledge, the dose of 1200 million cells used in this study represents the largest single dose of intravenous cell therapy administered in patients with stroke or in any other disease. The dose of 1200 million cells was chosen on the basis of findings of maximal efficacy with a cell dose of 12–15 million cells per kg in rodent stroke models and extrapolating the cell dose to human beings on the basis of differences in body mass between species. A similar sized, randomised, placebo-controlled clinical trial[34] of 120 patients that administered a mean dose of 280·75 million autologous bone marrow mononuclear cells at a median time window of 18·5 days after ischaemic stroke did not observe any beneficial effect, but noted that the cells were safe. Our early time window of 24–36 h with intravenous delivery is complementary to other stereotactic intracerebral transplantation approaches that target stroke and also seem promising.[6,7]

The limitations of this trial include the relatively small sample size and the expansion of the time window from 36–48 h that might have diluted the efficacy effect. Because we were not able to quantitate spleen size, we were unable to determine the effect of multipotent adult progenitor cell treatment on spleen size. Moreover, our measurement of white blood cells and inflammatory biomarkers were limited to measurements in the serum instead of in organs such as the spleen. The potential beneficial activity associated with early treatment noted in post-hoc analyses needs to be confirmed in subsequent trials. Further clinical trials in an earlier time window after stroke (<36 h) are planned (NCT02961504).

Contributors

DCH and LRW wrote the first draft of the manuscript, were involved in study design, data interpretation, and enrolled patients. WMC, SIS, GAF, DC, DRY, KU, DSL, APA, SS, and CAS enrolled patients, were involved in data interpretation, reviewed the manuscript, edited, and helped with revisions. WMC, SIS, GAF, and CAS were involved in study design. JDV did the statistical analysis. RWM was involved in study design, data interpretation, and writing of the manuscript.

Declaration of interests

DCH received grants from Athersys, payments to his university from Medpace for patient enrolment, has a patent on the MultiStem cells through his university and has received licensing revenue through his university. LRW received grants from SanBio and Athersys, and personal fees from SanBio. GAF is a consultant for Athersys; received personal fees from Medpace; and payment from Medpace to his institution for study costs. SS received grants from Athersys. SIS received grants from Athersys, and consulting fees that were paid to the institution from Mesoblast, Aldagen, and Celgene. CAS received grants from Athersys. DC received grants from Athersys. DSL received grants from the National Institutes of Health and National Institute of Neurological Disorders and Stroke and is a consultant for Stryker and Medtronic. DRY is a member of the Steering Committee of the RECOVER-Stroke Trial and received personal fees from Cyomedix and Aldagen. RWM is a co-founder of Athersys Inc, an employee of Athersys Inc, and holds patents. WMC, KU, APA, and JDV declare no competing interests.

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