JAMA Neurology | **Original Investigation**

Allogeneic Stem Cell Therapy for Acute Ischemic Stroke
The Phase 2/3 TREASURE Randomized Clinical Trial

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IMPORTANCE Cell therapy is a promising treatment approach for stroke and other diseases. However, it is unknown whether MultiStem (HLCM051), a bone marrow–derived, allogeneic, multipotent adult progenitor cell product, has the potential to treat ischemic stroke.

OBJECTIVE To assess the efficacy and safety of MultiStem when administered within 18 to 36 hours of ischemic stroke onset.

DESIGN, SETTING, AND PARTICIPANTS The Treatment Evaluation of Acute Stroke Using Regenerative Cells (TREASURE) multicenter, double-blind, parallel-group, placebo-controlled phase 2/3 randomized clinical trial was conducted at 44 academic and clinical centers in Japan between November 15, 2017, and March 29, 2022. Inclusion criteria were age 20 years or older, presence of acute ischemic stroke (National Institutes of Health Stroke Scale [NIHSS] score of 8-20 at baseline), confirmed acute infarction involving the cerebral cortex and measuring more than 2 cm on the major axis (determined with diffusion-weighted magnetic resonance imaging), and a modified Rankin Scale (mRS) score of 0 or 1 before stroke onset. Data analysis was performed between May 9 and August 15, 2022.

EXPOSURE Patients were randomly assigned to either intravenous MultiStem in 1 single unit of 1.2 billion cells or intravenous placebo within 18 to 36 hours of ischemic stroke onset.

MAIN OUTCOMES AND MEASURES The primary end points were safety and excellent outcome at day 90, measured as a composite of a modified Rankin Scale (mRS) score of 1 or less, a NIHSS score of 1 or less, and a Barthel index of 95 or greater. The secondary end points were excellent outcome at day 365, mRS score distribution at days 90 and 365, and mRS score of 0 to 1 and 0 to 2 at day 90. Statistical analysis of efficacy was performed using the Cochran-Mantel-Haenszel test.

RESULTS This study included 206 patients (104 received MultiStem and 102 received placebo). Their mean age was 76.5 (range, 35-95) years, and more than half of patients were men (112 [54.4%]). There were no between-group differences in primary and secondary end points. The proportion of excellent outcomes at day 90 did not differ significantly between the MultiStem and placebo groups (12 [11.5%] vs 10 [9.8%], P = .90; adjusted risk difference, 0.5% [95% CI, −7.3% to 8.3%]). The frequency of adverse events was similar between treatment groups.

CONCLUSIONS AND RELEVANCE In this randomized clinical trial, intravenous administration of allogeneic cell therapy within 18 to 36 hours of ischemic stroke onset was safe but did not improve short-term outcomes. Further research is needed to determine whether MultiStem therapy for ischemic stroke has a beneficial effect in patients who meet specific criteria, as indicated by the exploratory analyses in this study.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT02961504

+ **Supplemental content**

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S troke is the second leading global cause of death and a major cause of disability, with 6.6 million deaths attributed to stroke in 2019.[1] Evidence-based reperfusion therapies, including intravenous thrombolysis[2,3] and mechanical thrombectomy (MT),[4] are widely used for stroke treatment. Despite varied outcomes with these treatments, approximately 50% of patients still experience disability 3 months after stroke,[4] underlining the complexity and ongoing debate surrounding this therapeutic approach. This dynamic landscape highlights the need for new treatment options and expansion of the therapeutic window.

Cell therapy shows promise[5] in enhancing functional recovery by modulating the immune response, providing neuroprotection,[6] and restoring neural circuits in the injured brain.[7] Various cell types (mesenchymal stem cells, bone marrow mononuclear cells,[8] neural stem cells,[9] and induced pluripotent stem cells[7,10]) and administration routes (intravenous, intra-arterial, and intracerebral)[11-13] have been explored in different time windows (hours to months).[4,14]

Previous randomized clinical trials (RCTs) of autologous cell therapies for subacute and chronic stroke suggested improved outcomes pertaining to disability and activities of daily living.[15-18] However, these trials did not focus on acute stroke, had small sample sizes, and used time-consuming procedures for stem cell preparation and administration.

Allogeneic stem cells offer promising advantages over autologous stem cells for therapy because they have off-the-shelf properties and can be used early in the disease course.[19] MultiStem is a mass-produced stem cell product that provides benefits through various mechanisms. For example, MultiStem reduces inflammation, modulates immune dysregulation, protects damaged cells, and promotes angiogenesis, tissue repair, and healing.[20] Currently, few RCTs have examined the use of allogeneic stem cells, such as MultiStem, for stroke treatment.[19,21] The MultiStem in Acute Stroke Treatment to Enhance Recovery (MASTERS) trial used an allogenic, bone marrow–derived, clinical-grade, multipotent adult progenitor cell line and provided valuable insights, albeit within a 24- to 48-hour poststroke window.[19]

This study reports findings of the phase 2/3 Treatment Evaluation of Acute Stroke Using Regenerative Cells (TREASURE) trial, which explored MultiStem safety and efficacy for patients treated within 18 to 36 hours after ischemic stroke onset.

Methods

This multicenter, double-blind, parallel-group, placebo-controlled RCT (ClinicalTrials.gov: NCT02961504) was conducted in 44 academic and clinical centers across Japan. The TREASURE rationale, design, and methods were reported previously.[22] The trial protocol and statistical analysis plan are presented in Supplement 1. The TREASURE trial was approved by relevant local ethics committees and institutional review boards at each participating site. All patients or their representatives provided written informed consent before enrollment. The study followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

Key Points

Question Is intravenous allogeneic multipotent adult progenitor cell (MultiStem) therapy safe and effective for patients with acute ischemic stroke?

Findings In this randomized clinical trial with 206 participants, intravenous administration of MultiStem therapy within 18 to 36 hours of ischemic stroke onset was safe but did not improve short-term outcomes at 90 days compared with placebo. There were no grade 3 or 4 allergic reactions, including in older patients.

Meaning The results of this study support the safety of MultiStem, but further research is needed to determine whether MultiStem therapy for ischemic stroke has a beneficial effect in patients who meet specific criteria.

An independent data safety monitoring board evaluated the safety data after approximately 30% and 60% of the planned number of participants completed the 7-day follow-up. Bioimaging assessments were independently evaluated by a core assessment board via diffusion-weighted magnetic resonance imaging (DW-MRI) at baseline. All investigators had access to the data, and all authors vouch for the accuracy and completeness of the data, adherence to the trial protocol and statistical analysis plan (Supplement 1), and completeness of adverse event reporting.

Patients

The inclusion criteria were as follows: age 20 years or older, history of ischemic stroke with a persistent neurologic deficit (corresponding to a National Institutes of Health Stroke Scale [NIHSS] score of 8-20 at baseline), confirmed acute infarction involving the cerebral cortex and measuring more than 2 cm on the major axis (as determined with DW-MRI), and a modified Rankin Scale (mRS) score of 0 or 1 before stroke onset. We excluded patients with lacunar or brainstem infarction and a change in NIHSS score of 4 or greater during a minimum period of 6 hours between screening and randomization, and we also excluded those who received combined reperfusion therapy (intravenous thrombolysis plus MT). Patients who received only 1 reperfusion modality were included. The inclusion and exclusion criteria are summarized in eTable 1 in Supplement 2.
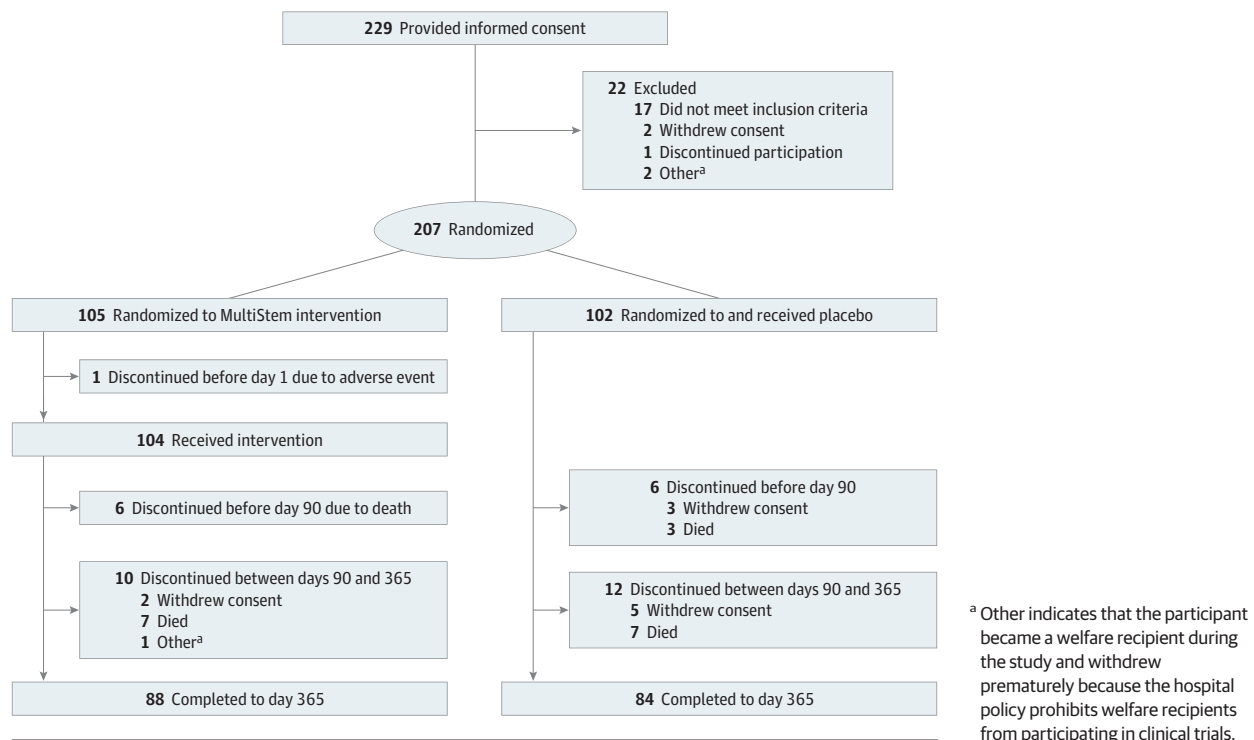
Randomization and Masking

A computer-generated randomization process was adopted to randomly assign patients (1:1) to receive MultiStem (HLCM051; Lonza) or placebo treatment. Randomization was stratified according to baseline NIHSS score (≤12 or ≥13), receipt of reperfusion therapy for acute ischemic stroke (yes or no), and age (≤64 or ≥65 years).

After 200 patients were randomized, the inclusion criterion for age was amended to reflect data safety monitoring board recommendations. An upper limit of 84 years was established because the 4 patients who died within 7 days of stroke onset were aged older than 84 years.

At each site, a designated staff member from a pharmacy or equivalent facility who was unblinded to the treatment assignment contacted an interactive web-response system

Figure 1. Trial Profile

- **229** Provided informed consent
  - **22** Excluded
    - **17** Did not meet inclusion criteria
    - **2** Withdrew consent
    - **1** Discontinued participation
    - **2** Other[a]
- **207** Randomized

- **105** Randomized to MultiStem intervention
  - **1** Discontinued before day 1 due to adverse event
  - **104** Received intervention
  - **6** Discontinued before day 90 due to death
  - **10** Discontinued between days 90 and 365
    - **2** Withdrew consent
    - **7** Died
    - **1** Other[a]
  - **88** Completed to day 365

- **102** Randomized to and received placebo
  - **6** Discontinued before day 90
    - **3** Withdrew consent
    - **3** Died
  - **12** Discontinued between days 90 and 365
    - **5** Withdrew consent
    - **7** Died
  - **84** Completed to day 365

[a] Other indicates that the participant became a welfare recipient during the study and withdrew prematurely because the hospital policy prohibits welfare recipients from participating in clinical trials.

vendor to access the assigned treatment of each patient and prepare the product accordingly. Patients and all trial personnel, including investigators and clinicians, were blinded to the treatment assignment. An opaque cover and sleeve were used over the intravenous infusion bag and tubing.

Procedures

The investigational product (both MultiStem and placebo) was sent to each trial site and stored appropriately by the pharmacy or relevant facility department. MultiStem was provided in a single unit containing 1.2 billion cells (±20%), which was cryopreserved in a medium comprising Plasma-Lyte A (for dilution), dimethylsulfoxide, and human serum albumin. The placebo consisted of only cryopreservation medium. Both MultiStem and placebo were supplied separately in 6-mL vials and sealed securely. The investigational product required storage at temperatures below −140 °C in a designated facility.

When needed for administration, appropriate trial personnel thawed the investigational product and prepared it for infusion. The product was then delivered to the department responsible for administration, following specific procedures.

Patients received a single intravenous dose of either MultiStem or placebo under gravity flow, which lasted 30 to 60 minutes and occurred between 18 and 36 hours after stroke onset.[22] Patient visits were scheduled at days 7, 30, 90, and 365 after randomization. Furthermore, patients were contacted via telephone at day 60 and every 2 months after day 90. Brain DW-MRI was performed at baseline. The central institution acquired brain MRI images obtained during the screening process, and researchers evaluated infarct volume

and Alberta Stroke Program Early Computed Tomography score independently under blinded status.

Efficacy Assessment

The primary efficacy outcome was the proportion of patients with an excellent outcome at day 90. This was defined as meeting the following composite scoring criteria: mRS score of 1 or less (range, 0-6), NIHSS total score of 1 or less (range, 0-42), and Barthel index (BI) score of 95 or greater (range, 0-100).

The key secondary end points were the proportion of patients with an excellent outcome at day 365, distribution of mRS scores at days 90 and 365, and proportion of patients with mRS scores of 0 to 1 and 0 to 2 at day 90. eTable 2 in Supplement 2 presents a complete list of secondary parameters.

Safety Assessment

The safety assessment comprised several primary safety end points. These were as follows: grade 3 or 4 infusion-related reactions (Common Terminology Criteria for Adverse Events, version 4.0), including cardiovascular and respiratory function abnormalities or allergic reactions occurring within 24 hours after infusion; serious adverse events occurring within 7 days after treatment; worsening of neurological symptoms, defined as an increase of 4 or more points in NIHSS score vs baseline assessed through 7 days after treatment related to the investigational product; death or life-threatening adverse events up to day 90; and secondary infection up to day 90.

Statistical Analysis

Based on the tissue plasminogen activator (t-PA) and MT data from the MASTERS trial,[19] we estimated that including 100

patients per group would yield 90% power at a 5% significance level to detect a treatment effect of the primary efficacy outcome. The primary efficacy outcome effect sizes were assumed to be 18.5% and 3.8% in the MultiStem and placebo groups, respectively. We adjusted the number of patients to 110 per group to account for possible exclusions or dropouts. Efficacy analyses were based on the full analysis set, including patients who underwent randomization and mRS, NIHSS, and BI assessments at least once after day 7. Safety outcomes were evaluated in the safety analysis set, which included all randomized and treated participants. The superiority of the MultiStem treatment to placebo was evaluated using the Cochran-Mantel-Haenszel (CMH) test (with a 2-tailed significance level of .05), adjusted for baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (yes or no), and age (≤74 or ≥75 years). The mRS score distribution was summarized with counts and percentages at days 90 and 365 and was compared between groups using the CMH test with modified ridit scores and stratified by baseline adjustment factors. Exploratory subgroup analyses of mRS scores of 2 or less at day 90 with no correction for multiple comparisons were conducted using the CMH test with adjustments for subgroups of age (≤64, ≥65, ≤74, or ≥75 years) and stroke volume (<25, ≥25, <50, ≥50, <70, or ≥70 mL). These subgroup analyses were defined in the statistical analysis plan (version 1.0) finalized before trial unmasking (Supplement 1).

To evaluate the long-term effects of MultiStem, a post hoc analysis was conducted for the proportion of patients with global stroke recovery at day 365. This analysis comprised a mRS score of 2 or less, an NIHSS score improvement of 75% or greater, and a BI score of 95 or greater.

Patients who missed the evaluation at day 90 were assessed using their last postrandomization primary efficacy assessment (day 7 or later) carried forward (LOCF) to impute the missing day 90 assessment. For the secondary end points (ie, excellent outcome at day 365 and distribution of mRS scores at days 90 and 365) and post hoc analysis of global stroke recovery, BI score of 95 or greater, and mRS score of 2 or less at day 365, missing data were imputed in a similar manner. Sensitivity analysis for missing data included evaluation of excellent outcomes at days 90 and 365 for observed patients using the CMH test. Other secondary efficacy data were not imputed. Patients who died during the study were assigned a mRS score of 6, a binary NIHSS score of greater than 1, and a BI score of less than 95 at all subsequent time points.

In all efficacy analyses, we used age 74 years or younger or 75 years or older as an adjusted factor (instead of age ≤64 and ≥65 years as a randomization factor) in the CMH test, with blind review and redefinition of the primary analysis in the revised protocol with Pharmaceuticals and Medical Devices Agency consultation before unmasking.

In this study, statistical multiplicity was not considered for the analysis of secondary end points, subgroup analysis, and post hoc analysis. All data analyses were performed with SAS, version 9.4, and with SAS/STAT, version 14.2 (SAS Institute). Data analysis was performed between May 9 and August 15, 2022.

Table 1. Patient Demographic and Baseline Characteristics

Characteristic	Value[a]	
	MultiStem (n = 104)	Placebo (n = 102)
Sex		
Male	56 (53.8)	56 (54.9)
Female	48 (46.2)	46 (45.1)
Age, y		
Mean (SD)	76.7 (10.4)	76.2 (10.6)
Median (range)	79 (40-95)	78 (35-95)
≥65	94 (90.4)	90 (88.2)
≥75	66 (63.5)	66 (64.7)
Patients with left hemisphere events	61 (58.7)	42 (41.2)
Patients who received reperfusion therapy		
Yes	56 (53.8)	52 (51.0)
Tissue plasminogen activator	24 (23.1)	12 (11.8)
Mechanical thrombectomy	32 (30.8)	40 (39.2)
No	48 (46.2)	50 (49.0)
NIHSS score		
Mean (SD)	13.7 (3.9)	13.9 (3.9)
Median (range)	14 (8-20)	14 (8-20)
≤12	41 (39.4)	37 (36.3)
Infarct volume, mL[b]		
Mean (SD)	42.0 (48.4)	54.3 (57.0)
Median (range)	26.5 (0.1-308.9)	39.7 (0.9-423.5)

Abbreviation: NIHSS, National Institutes of Health Stroke Scale.

[a] Unless indicated otherwise, values are presented as number (percentage) of patients.

[b] The number of patients for the MultiStem and placebo groups was 101 and 97, respectively.

Results

A total of 229 patients were recruited between November 15, 2017, and March 30, 2021, with follow-up to day 365 on March 29, 2022. Among them, 207 patients were randomized to treatment: 105 to MultiStem and 102 to placebo. One patient discontinued participation before day 1. Thus, 206 patients received an intravenous infusion of MultiStem (n = 104) for 33 to 72 minutes or placebo (n = 102) for 30 to 77 minutes and were included in the safety analysis and full analysis sets (**Figure 1**). More than half of patients were men (112 [54.4%]), and 94 (45.6%) were women; the mean age of patients was 76.5 (range, 35-95) years. Before day 90 after dosing, 6 patients from each group withdrew.

Table 1 presents patient demographics and baseline characteristics. This study included a high proportion of older patients, with a median age of 79 (range, 40-95) years in the MultiStem group and 78 (range, 35-95) years in the placebo group. Proportions of patients who received reperfusion therapy, mean NIHSS scores, and infarct volumes were similar between groups.

The rate of excellent outcomes at day 90 did not differ significantly between the MultiStem and placebo groups (12 [11.5%] vs 10 [9.8%], *P* = .90; adjusted risk difference, 0.5% [95% CI, −7.3% to 8.3%]). Furthermore, there were no

Table 2. Primary and Secondary Efficacy End Points

	Day 90				Day 365			
	No. (%)		Adjusted risk difference, % (95% CI)	P value	No. (%)		Adjusted risk difference, % (95% CI)	P value
End point	MultiStem (n = 104)	Placebo (n = 102)			MultiStem (n = 104)	Placebo (n = 102)		
Excellent outcome								
Imputation[a,b]	12/104 (11.5)	10/102 (9.8)	0.5 (−7.3 to 8.3)	.90	16/104 (15.4)	11/102 (10.8)	3.4 (−5.0 to 11.8)	.43
Observed[a,c]	12/103 (11.7)	10/98 (10.2)	0.4 (−7.6 to 8.4)	.92	14/97 (14.4)	11/93 (11.8)	0.4 (−8.1 to 8.9)	.93
mRS score distribution[b,d]								
0	5 (4.8)	3 (2.9)	NA		9 (8.7)	1 (1.0)	NA	
1	9 (8.7)	8 (7.8)	NA		11 (10.6)	13 (12.7)	NA	
2	20 (19.2)	12 (11.8)	NA		18 (17.3)	13 (12.7)	NA	
3	15 (14.4)	17 (16.7)	NA	.62	15 (14.4)	17 (16.7)	NA	.42
4	31 (29.8)	39 (38.2)	NA		22 (21.2)	33 (32.4)	NA	
5	18 (17.3)	20 (19.6)	NA		16 (15.4)	15 (14.7)	NA	
6	6 (5.8)	3 (2.9)	NA		13 (12.5)	10 (9.8)	NA	
Global stroke recovery[c]	20/96 (20.8)	16/94 (17.0)	0.9 (−9.0 to 10.9)	.86	NA	NA	NA	NA
mRS score[c]								
≤1	14/97 (14.4)	11/95 (11.6)	1.1 (−7.7 to 9.9)	.81	NA	NA	NA	NA
≤2	34/97 (35.1)	23/95 (24.2)	8.3 (−3.6 to 20.2)	.17	NA	NA	NA	NA
NIHSS score[c]								
≤1	19/85 (22.4)	19/85 (22.4)	−2.3 (−13.9 to 9.3)	.71	NA	NA	NA	NA
Improvement ≥75%[c]	30/85 (35.3)	29/85 (34.1)	−1.6 (−15.4 to 12.2)	.82	NA	NA	NA	NA
Barthel index ≥95[c]	31/97 (32.0)	24/93 (25.8)	3.7 (−8.0 to 15.3)	.54	NA	NA	NA	NA

Abbreviations: CMH, Cochran-Mantel-Haenszel; mRS, modified Rankin Scale; NA, not applicable; NIHSS, National Institutes of Health Stroke Scale.

[a] Treatments were compared using the CMH test adjusted for baseline NIHSS score (≤12 or ≥13), receipt of concomitant reperfusion therapy (yes or no), and age (20-74 or ≥75 years). Risk differences and corresponding 2-sided 95% CIs between the MultiStem and placebo groups were adjusted for the same factors applied in the CMH test and calculated using the Mantel-Haenszel method by Sato T.

[b] Imputation was performed by last postrandomization efficacy assessment

of excellent outcome and mRS score carried forward.

[c] Observed cases. Regarding excellent outcome, included death cases as worst case and available partial missing case (ie, the case in at least available 1 of 3 composite scoring criteria for excellent outcome) of composite scoring criteria for excellent outcome.

[d] P values were calculated using the CMH test adjusted for baseline NIHSS score (≤12 or ≥13), receipt of concomitant reperfusion therapy (yes or no), and age (20-74 or ≥75 years) using modified ridit scores.

statistically significant between-group differences for any of the secondary end points (**Table 2**). The mRS score distribution at days 90 and 365 is presented in **Figure 2**.

The proportions of missing assessments, defined as the difference in total patient numbers between LOCF data and observed patient data, for excellent outcomes at day 90 were 1 of 104 (1.0%) and 4 of 102 (3.9%) in the MultiStem and placebo groups, respectively (Table 2). Similarly, at day 365, the proportions of missing assessments for excellent outcomes were 7 of 104 (6.7%) and 9 of 102 (8.8%) in these groups. The proportions of missing data for the primary end point assessment were low in both treatment groups.

Exploratory subgroup analyses of mRS scores of 2 or less at day 90 with no correction for multiple comparisons were conducted for the age and ischemic core volume subgroups. Patients with an ischemic core volume of 50 mL or less had significantly better outcomes in the MultiStem vs placebo groups (8 of 27 [29.6%] vs 3 of 37 [8.1%], P = .04; adjusted risk difference, 20.4% [95% CI, 1.0% to 39.9%]). Patients aged 64 years or younger also tended to have better outcomes in the MultiStem vs placebo groups, although the difference between groups was not statistically significant (8 of 10 [80.0%] vs 5 of 12 [41.7%], P = .08; adjusted risk difference, 37.2% [95% CI, −0.4% to 74.8%]; **Figure 3**). Although the results of subgroup analyses had

potential statistical multiplicity, these beneficial effects were not observed in patients with an ischemic core volume of less than 50 mL for both the MultiStem vs placebo groups (26 of 67 [38.8%] vs 20 of 54 [37.0%], P = .87; adjusted risk difference, −1.4% [95% CI, −16.9% to 14.2%]), nor were they observed for patients with an ischemic core volume of less than 25 mL (17 of 46 [37.0%] vs 14 of 32 [43.8%], P = .70; adjusted risk difference, −4.3% [95% CI, −25.6% to 17.1%]), 25 mL or greater (17 of 48 [35.4%] vs 9 of 59 [15.3%], P = .11; adjusted risk difference, 11.7% [95% CI, −3.2% to 26.6%]), less than 70 mL (29 of 80 [36.3%] vs 22 of 66 [33.3%], P = .85; adjusted risk difference, 1.4% [95% CI, −12.6% to 15.3%]), or 70 mL or greater (5 of 14 [35.7%] vs 1 of 25 [4.0%], P = .05; adjusted risk difference, 25.4% [95% CI, −2.2% to 52.9%]). Beneficial effects also were not observed for patients aged 65 years or older (26 of 87 [29.9%] vs 18 of 83 [21.7%], P = .23; adjusted risk difference, 7.7% [95% CI, −4.8% to 20.3%]), 74 years or younger (18 of 37 [48.6%] vs 12 of 35 [34.3%], P = .42; adjusted risk difference, 8.7% [95% CI, −13.1% to 30.5%]), or 75 years or older (16 of 60 [26.7%] vs 11 of 60 [18.3%], P = .27; adjusted risk difference, 8.0% [95% CI, −5.8% to 21.9%]).

eTable 3 in Supplement 2 presents the results of exploratory post hoc analyses of proportions of patients in the MultiStem group with global stroke recovery and a BI score of

95 or greater at day 365 with no correction for multiple comparisons, which were better than those in placebo group. For global stroke recovery, 29 patients (27.9%) in the Multi-Stem group and 16 (15.7%) in the placebo group had improvement (adjusted risk difference, 11.0% [95% CI, 0.8% to 21.3%]; $P = .04$). For BI scores of 95 or greater, 37 patients (35.6%) in the MultiStem group and 23 (22.5%) in the placebo group had higher scores (adjusted risk difference, 11.3% [95% CI, 0.2% to 22.4%], $P = .05$).

The primary safety end points including grade 3 or 4 infusion-related allergic reactions did not differ between groups (eTable 4 in Supplement 2). Treatment-emergent adverse events (eTable 5 in Supplement 2) related to the study drug occurred in 31 MultiStem-treated patients (29.8%) and 12 placebo-treated patients (11.8%).

Discussion

To our knowledge, this is the first double-blind phase 2/3 RCT to evaluate cell therapy for acute ischemic stroke. Previous stem cell therapy RCTs used autologous stem cells,[15-18] rendering double-blind studies challenging. Because multi-center RCTs of allogeneic cells in acute stroke are limited,[19,21] this study has substantial potential to impact novel treatments for ischemic stroke.

Although there were no significant differences in the primary and secondary end points between the MultiStem and placebo groups in this study, exploratory subgroup analyses with no correction for multiple comparisons conducted with patients with mRS scores of 0 to 2 at day 90 seemed to show better outcomes in the MultiStem group, particularly for patients with ischemic core volumes of 50 mL or greater and those aged 64 years or younger. Exploratory post hoc analyses with no correction for multiple comparisons indicated significantly higher proportions of patients with global stroke recovery and a BI of 95 or greater at day 365 in the MultiStem vs placebo groups. The occurrence of adverse events was comparable between groups.

Contrary to our hypothesis, MultiStem did not improve clinical outcomes as expected. Previous post hoc analysis of early treatment (<36 hours) in phase 2 of the MASTERS trial reported substantially increased rates of excellent outcomes at day 365 in the MultiStem group.[19] Additionally, another post hoc analysis of the MASTERS trial showed a higher rate of excellent outcomes in early treatment (<36 hours) excluding patients who received t-PA plus MT[19]; this exclusion criterion was also used in the TREASURE study.[22] The disparity in results between the MASTERS and TREASURE trials may be attributable to the inclusion of older patients, which may have masked the immediate effect of MultiStem treatment. However, a trend toward better outcomes was observed in patients aged younger than 64 years. The median age of TREASURE participants was 78 to 79 years, which was substantially higher than the age in almost all clinical stroke studies, including the previous MASTERS trial on MultiStem,[19] by more than 10 to 15 years. One potential reason may be Japan's aging population, as the median age of stroke in Japan is 74 (IQR, 66-82) years.[23] Interestingly, this age distribution

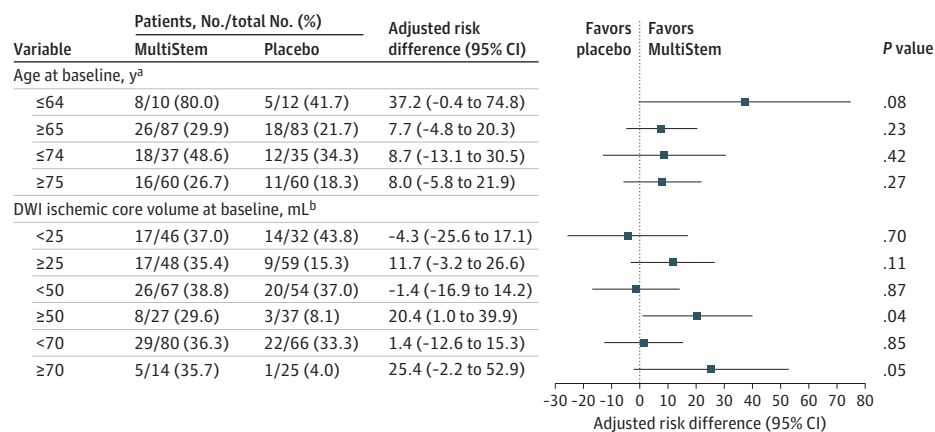**Figure 2. Distribution of Modified Rankin Scale Score on Days 90 and 365**
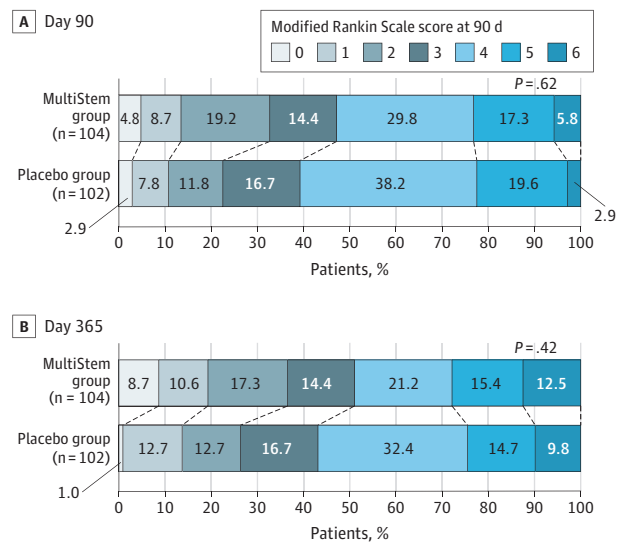
A and B, Modified Rankin Scale scores on days 90 (A) and 365 (B). Scores range from 0 to 6, with 0 indicating no symptoms; 1, symptoms without clinical disability; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability; and 6, death. Imputation was performed by last postrandomization primary efficacy assessment carried forward.

concurred with participants in the TREASURE study. Furthermore, based on the safety results of the MASTERS trial, no upper age limit was set at the beginning of the TREASURE trial.[19] The influence of the substantial number of older participants on the findings of this study remains uncertain. Exploration of the impact of MultiStem therapy on aging animals in future studies could provide valuable insights. Cell therapy aims to facilitate regeneration, repair, and plasticity of surviving neural tissues, which may require longer evaluation periods. The underlying mechanisms of MultiStem involve modulating the peripheral immune system and promoting a regenerative environment, which may contribute to long-term efficacy.[5,24] Results from the MASTERS trial at 1 year support improved outcomes in the MultiStem group compared with the control group, despite intravenously administered MultiStem disappearing from the body shortly after administration.[19] Our findings of a better trend in outcomes at 1 year, as determined by the exploratory post hoc analysis, aligns with the exploratory post hoc analysis of the MASTERS trial.[19]

Autologous stem cell therapy for stroke can improve long-term outcomes and survival.[16] Selim[25] proposed evaluating outcomes at day 365 for intracerebral hemorrhage trials to detect long-term treatment effects. Other cell therapies for cerebral infarction can restore corticospinal tract function up to 1 year after stem cell administration.[26] Applying this principle to interventions promoting neural repair in ischemic stroke seems beneficial.

In our exploratory subgroup analyses with no correction for multiple comparisons, MultiStem seemed to be effective when the cerebral infarction was 50 mL or greater. This is probably because smaller infarct volumes generally respond better to conventional therapy, and it can be challenging to detect the efficacy of cell therapy due to ceiling effects.[27] For patients with

Figure 3. Subgroup Analyses of Modified Rankin Scale Score Scores of 0 to 2 at Day 90 Stratified by Age and Ischemic Core Volume

Variable	Patients, No./total No. (%)		Adjusted risk difference (95% CI)	Favors placebo Favors MultiStem	P value
	MultiStem	Placebo			
Age at baseline, y[a]					
≤64	8/10 (80.0)	5/12 (41.7)	37.2 (−0.4 to 74.8)		.08
≥65	26/87 (29.9)	18/83 (21.7)	7.7 (−4.8 to 20.3)		.23
≤74	18/37 (48.6)	12/35 (34.3)	8.7 (−13.1 to 30.5)		.42
≥75	16/60 (26.7)	11/60 (18.3)	8.0 (−5.8 to 21.9)		.27
DWI ischemic core volume at baseline, mL[b]					
<25	17/46 (37.0)	14/32 (43.8)	−4.3 (−25.6 to 17.1)		.70
≥25	17/48 (35.4)	9/59 (15.3)	11.7 (−3.2 to 26.6)		.11
<50	26/67 (38.8)	20/54 (37.0)	−1.4 (−16.9 to 14.2)		.87
≥50	8/27 (29.6)	3/37 (8.1)	20.4 (1.0 to 39.9)		.04
<70	29/80 (36.3)	22/66 (33.3)	1.4 (−12.6 to 15.3)		.85
≥70	5/14 (35.7)	1/25 (4.0)	25.4 (−2.2 to 52.9)		.05

Adjusted risk difference (95% CI): −30 −20 −10 0 10 20 30 40 50 60 70 80

Risk differences and corresponding 2-sided 95% CIs in the MultiStem and placebo groups adjusted using the same factors in the Cochran-Mantel-Haenszel (CMH) test were calculated using the Mantel-Haenszel method by Sato T.

[a] Treatments were compared after categorizing patients by age at baseline using the CMH test adjusted for baseline National Institutes of Health Stroke Scale (NIHSS) score (≤12 or ≥13) and receipt of concomitant reperfusion therapy (yes or no). There were 97 patients in the MultiStem group and 95 in the placebo group.

[b] Treatments were compared after stratifying patients by diffusion-weighted imaging (DWI) ischemic core volume at baseline using the CMH test adjusted for baseline NIHSS score (≤12 or ≥13), receipt of concomitant reperfusion therapy (yes or no), and age (20-74 or ≥75 years) There were 94 patients in the MultiStem group and 91 in the placebo group.

large infarction volumes, thrombectomy may be less effective, leading to poor outcomes and increased intracranial hemorrhage, even after successful recanalization.[28] Although recent studies have demonstrated the efficacy of endovascular therapy for large infarctions, infarct volume remains a substantial factor in poor outcomes. Therefore, our finding that individuals with cerebral infarction of 50 mL or greater benefit from cell therapy holds crucial clinical implications, as these patients may not benefit from conventional treatments like thrombectomy.

In this study, MultiStem also demonstrated an acceptable safety profile. Our study findings align with the observations of Rasmusson et al,[29] as we did not encounter grade 3 or 4 allergic reactions associated with MultiStem infusions. Moreover, the MultiStem mechanism of action includes immunomodulation, and the absence of suspected serious adverse reactions within the first 7 days after administration further confirms the safety of this cell therapy approach.

Limitations

Patient heterogeneity is a limitation of this trial. The inclusion of patients with advanced age and posterior circulation involvement in stroke may have diluted the treatment effects. The decision to include such patients remains controversial, as previous RCTs of MT in this subgroup yielded varying results.[30-34] Statistical multiplicity was not considered for the subgroup analysis and post hoc analysis in this study. All of these results are exploratory, and most subgroup analyses had small sample sizes. Moreover, patients treated with both t-PA and MT were excluded. The MASTERS-2 clinical trial including patients treated with both t-PA and MT is being conducted.

Conclusions

In the TREASURE RCT, intravenous allogeneic cell therapy within 18 to 36 hours of ischemic stroke onset failed to demonstrate a short-term therapeutic effect. However, efficacy was suggested in patients with large infarcts and possibly in younger patients based on exploratory subgroup analyses with no correction for multiple comparisons. A combined analysis of the TREASURE and ongoing MASTERS-2 trials will be conducted to confirm the efficacy and safety of MultiStem therapy.

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Conflict of Interest Disclosures: Dr Houkin reported receiving personal fees from Healios KK during the conduct of the study and from Bayer Yakuhin, Idorsia Japan, and Daiichi Sankyo Co Ltd outside the submitted work. Dr Osanai reported receiving meeting and travel support from Healios KK outside the submitted work. Dr Uchiyama reported receiving personal fees from Healios KK during the conduct of the study and from Bayer and Boehringer Ingelheim outside the submitted work. Dr Minematsu reported receiving personal fees from Healios KK during the conduct of the study and lecture fees from Pfizer, Bristol Myers Squibb, Takeda, Bayer Yakuhin, Daiichi Sankyo Co Ltd, and Nippon Chemiphar outside the submitted work. Dr Hess reported receiving personal fees from Athersys Inc for advisory board service during the conduct of the study. In addition, Dr Hess reported holding a patent for multipotent progenitor cells in neurological disease with Augusta University and Athersys Inc, with royalties paid from St Marys on the Hill. No other disclosures were reported.

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