Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Inclusion and Exclusion Criteria

Inclusion criteria

Patients were considered eligible to participate in the trial if they met all of the following criteria:

1. Japanese male or female patients between 20 and 84 years of age.
2. Clinical diagnosis of ischemic stroke in the cerebral cortex.
3. Occurrence of an ischemic stroke with clear motor or speech deficit as documented by a National Institutes of Health Stroke Scale (NIHSS) score of 8–20 (in the baseline assessment) that did not change by ≥4 points from the time of screening to the baseline assessment.

   NOTE: The NIHSS assessment must be performed by the trial doctor who is trained for the NIHSS assessment.

   NOTE: The baseline and screening NIHSS assessments must be separated by at least 6 h.

   NOTE: The NIHSS score must be collected within 4 hours after reperfusion therapy for patients who received tissue plasminogen activator (tPA) treatment or underwent mechanical reperfusion.

   NOTE: The baseline NIHSS assessment must be confirmed within 16–34 h after the onset of ischemic stroke.
4. Onset of ischemic stroke within 18–36 hours prior to the start of administration of the investigational product.

   NOTE: Time of onset is defined as the time at which symptoms first began. For ischemic stroke that occurred during sleep, the time of onset is defined as the time when the patient was last observed to be normal or the latest time when the patient was self-reported to be normal.
5. Confirmation of hemispheric cortical infarct on brain magnetic resonance imaging (MRI) including diffusion-weighted imaging with b-value of 1000 demonstrating an acute lesion measuring ≥2.0 cm in the longest diameter.
6. A modified Rankin Scale (mRS) score of 0 or 1, obtained by either self-report or family report, prior to the onset of ischemic stroke.
7. Female patients who were:
   a. Not pregnant, not breastfeeding/interrupting breastfeeding, and not planning on becoming pregnant during the trial;
   b. Not of childbearing potential, defined as women who had been postmenopausal for at least 1 year, who had been surgically sterilized, or who underwent hysterectomy at least 3 months prior to the start of this trial; or
   c. If of childbearing potential, agreement to follow the investigators' advice and use an effective contraceptive method up to the first year of the trial. Effective contraceptive methods include contraceptive methods used consistently and correctly (oral contraceptives, intrauterine devices, diaphragm, or male or female condoms), abstinence, and a sterile sexual partner.

8. Male patients with female partners of childbearing potential must agree to follow the investigators' advice and use adequate contraceptive methods (the combination of a condom and another form of contraception) for the first year of the trial if engaging in sexual intercourse.
9. Patients or legal representatives must sign the informed consent form after the nature of the trial and disclosure of data have been explained.
10. Willing and able to comply with all aspects of the treatment and testing schedule.
11. Willing and able to return to the trial site for the post-treatment evaluations

Exclusion criteria

Patients were not eligible to participate in the trial if they met any of the following criteria:

1. Presence of a lacunar infarct, a lesion of ≤ 2.0 cm in the longest diameter, or a brainstem infarct on MRI as the etiology of ischemic stroke.
2. Reduced level of consciousness (score of 3 for item 1a of NIHSS).
3. Occurrence of hemorrhagic transformation as evidenced by computed tomography or brain MRI that is clinically significant in the opinion of the investigators.
4. Ipsilateral focal neurological deficits from prior lesions in the brain that would complicate evaluation.
5. Experience of seizures since the onset of ischemic stroke.
6. History of a neurological event such as stroke or clinically significant head trauma within 6 months prior to the start of screening.
7. Patients who both received tPA treatment and mechanical reperfusion (patients were eligible for the trial if they had only one such treatment).

   Note: Based on the results of post hoc analysis of the MASTERS trial, patients who both received tPA treatment and mechanical reperfusion were excluded. In the MASTERS trial, post hoc analysis of 27 patients in MultiStem group and 52 patients in placebo group, excluding both reperfusion treatments, showed that proportion of excellent outcome at Day 90 in MultiStem group (18.5%) was significantly higher than in placebo group (3.8%, *P*=.03)
8. Uncontrolled hypertension, defined as persistent systolic blood pressure > 220 mmHg or diastolic blood pressure > 120 mmHg, despite antihypertensive therapy.
9. Blood glucose level of <50 mg/dL or >350 mg/dL at baseline.
10. A significant comorbid medical condition, including:
    a. Severe kidney disease requiring hemodialysis or peritoneal dialysis,
    b. Advanced liver disease such as hepatitis or liver cirrhosis,
    c. Severe congestive heart failure or history of ejection fraction < 30%,
    d. Severe lung disease requiring home oxygen, and
    e. Active unstable angina requiring daily treatment with nitrates or other medications.
11. Known human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or an immunocompromised status.

12. Alzheimer's disease or other dementias, Parkinson's disease, or any other neurological disorder that in the opinion of the trial doctor would affect the ability to participate in the trial or confound study assessments.
13. History of malignant tumor(s) within 2 years of the onset of ischemic stroke, with the exception of adequately treated basal or squamous cell carcinoma of the skin.
14. Contraindication for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, excessive body weight, or claustrophobia.
15. Thrombocytopenia (platelet count < 100,000/mm^3) or heparin-induced thrombocytopenia,
16. Known allergy to human tissue or bovine or porcine products or religious objections to biological products.
17. Prior participation in another clinical trial involving investigational pharmacological agents or devices within 30 days prior to providing consent to receive the investigational product or participation in pharmacological agents, devices, or investigational rehabilitation stroke recovery program is planned during the trial,
18. Other serious medical or psychiatric disorders that is not adequately controlled that in the investigators' opinion would not permit the patient to be managed according to the protocol.
19. Previous surgical removal of the spleen.
20. Major fluctuation in neurological status after the onset of ischemic stroke indicating progression or expansion of ischemic stroke or possible transient ischemic attack,
21. Plan to undergo a neurovascular procedure (e.g., carotid endarterectomy, stent placement) within the first year following ischemic stroke.
22. Abnormal laboratory test results that investigators consider clinically significant and inappropriate for the trial.

eTable 2. List of Secondary Efficacy End Points

1.	**Endpoints**
2.	The proportion of patients with an excellent outcome defined by the functional assessments on day 365.
3.	The proportion of patients exhibiting functional outcomes throughout the range of mRS scores by shift analysis on day 90.
4.	The proportion of patients exhibiting functional outcomes throughout the range of mRS scores by shift analysis on day 365.
5.	The proportion of patients with an mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and a BI of ≥95 on 90.
6.	The proportions of patients with an mRS score of ≤1 and ≤2 on day 90.
7.	The proportion of patients with an NIHSS score of ≤1 on day 90.
8.	The proportion of patients with a favorable outcome (NIHSS score improvement of ≥75% from baseline) for neurological symptoms on day 90.
9.	The proportion of patients with a BI of ≥95 on day 90.
10.	The proportion of patients who survived without life-threatening adverse events (AEs) on day 90.
11.	The proportion of patients who survived without secondary infections on day 90.
12.	Dichotomous assessment on Day 90. Integrated assessment using three efficacy endpoints (mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and BI of ≥95) and two safety endpoints (survival without life-threatening AEs and secondary infections).

Abbreviations: BI, Barthel index; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale

eTable 3. Efficacy End Points in the Post Hoc Analysis

	Day 365			
	MultiStem (n = 104)	Placebo (n = 102)	p-value	Adjusted risk difference (%) [95% CI]
Global stroke recovery	29 (27.9)	16 (15.7)	.04	11.0 [0.8, 21.3]
mRS ≤ 2	38 (36.5)	27 (26.5)	.15	8.7 [-3.1, 20.4]
Barthel index ≥ 95	37 (35.6)	23 (22.5)	.05	11.3 [0.2, 22.4]

Abbreviations: mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale
Data are presented as n (%).
Treatments were compared using the Cochran–Mantel–Haenszel (CMH) test adjusted for the baseline NIHSS score (≤12 or ≥13), receipt of concomitant reperfusion therapy (Yes or No), and age (20–74 or ≥75). The risk difference and corresponding two-sided 95% confidence interval (CI) between the groups adjusted for the same factors used in the CMH test were calculated using the Mantel–Haenszel method by Sato T.
Imputation was performed by last post-randomization efficacy assessment of Global stroke recovery, mRS ≤ 2, and Barthel index ≥ 95 carried forward.

eTable 4. Safety End Points

	No. (%)	
	MultiStem (n=104)	Placebo (n=102)
Grade 3 or 4 infusion-related allergic reactions related to the investigational product[a]	0 (0.0)	0 (0.0)
SAEs occurring within 7 days after treatment related to the investigational product	0 (0.0)	1 (1.0)
Worsening of neurological symptoms related to the investigational product[b]	0 (0.0)	1 (1.0)
Death, life-threatening AEs on day 90	7 (6.7)	6 (5.9)
Incidence of secondary infections on day 90	48 (46.2)	38 (37.3)

Abbreviations: SAE, serious adverse event; AE, adverse event.
[a] Abnormality of cardiovascular or respiratory function or allergic reactions (e.g., rash, erythema) occurring within 24 h after treatment.
[b] Worsening was defined as an increase of ≥4 points in the National Institutes of Health Stroke Scale score versus baseline assessed through 7 days after treatment.

eTable 5. Treatment-Emergent Adverse Events

	MultiStem (n = 104)	Placebo (n = 102)
TEAEs	104 (100.0)	101 (99.0)
Study drug related TEAEs	31 (29.8)	12 (11.8)
Infusion-related allergic reaction	8 (7.7)	2 (2.0)
Grade 1: Mild	4 (3.8)	2 (2.0)
Grade 2: Moderate	4 (3.8)	0 (0.0)
Grade 3: Severe	0 (0.0)	0 (0.0)
Grade 4: Life-threatening	0 (0.0)	0 (0.0)
Grade 5: Death	0 (0.0)	0 (0.0)
Event leading to permanent discontinuation of investigational product infusion	0 (0.0)	0 (0.0)
Serious TEAEs	42 (40.4)	47 (46.1)
Event leading to death	14 (13.5)	10 (9.8)

Abbreviations: TEAE, treatment-emergent adverse event
Data are presented as n (%).