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Randomized placebo-controlled trial of CL2020, an allogenic muse cell–based product, in subacute ischemic stroke

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Abstract

Effective treatments for stroke after the acute phase remain elusive. Muse cells are endogenous, pluripotent, immune-privileged stem cells capable of selectively homing to damaged tissue after intravenous injection and replacing damaged/lost cells via differentiation. This randomized, double-blind, placebo-controlled trial enrolled ischemic stroke patients with modified Rankin Scale (mRS) $\geq$3. Randomized patients received a single intravenous injection of an allogenic Muse cell-based product, CL2020 (n = 25), or placebo (n = 10), without immunosuppressant, 14–28 days after stroke onset. Safety (primary endpoint: week 12) and efficacy (mRS, other stroke-specific measures) were assessed up to 52 weeks. Key efficacy endpoint was response rate (percentage of patients with mRS $\leq$2 at week 12). To week 12, 96% of patients in the CL2020 group experienced adverse events and 28% experienced adverse reactions (including one Grade 4 status epilepticus), compared with 100% and 10%, respectively, in the placebo group. Response rate was 40.0% (95% CI, 21.1–61.3) in the CL2020 group and 10.0% (0.3–44.5) in the placebo group; the lower CI in the CL2020 group exceeded the preset efficacy threshold (8.7% from registry data). This randomized placebo-controlled trial demonstrated CL2020 is a possible effective treatment for subacute ischemic stroke.

Registry information: JAPIC Clinical Trials Information site (JapicCTI-184103, URL: https://www.clinicaltrials.jp/cti-user/trial/ShowDirect.jsp?japicId=JapicCTI-184103).

Keywords

Clinical trials, ischemic stroke, muse cells, regeneration therapy, stem cells

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Introduction

Stroke is the second most common cause of death and disability worldwide.[1] Although advances in acute-phase treatment have reduced mortality, population growth and longer lives have led to more stroke survivors, estimated at 80 million in 2016.[1] After the acute phase, treatment for subacute stroke is limited to rehabilitation, which reaches its maximal effect at 3–6 months, after which patients live with burdensome sequelae.[2] Therefore, treatments that are effective in the subacute stage are needed.

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Ideally, treatment for subacute stroke would replenish lost neural cells and reconstruct neural circuits; stem cells may fulfill this purpose. Various stem cell types, including mesenchymal stem cells (MSCs) from bone marrow (BM), mononuclear cells from BM (BM-MNCs), and umbilical cord blood, have been trialed in stroke and represent a promising strategy.[3,4] However, no randomized placebo-controlled trial has demonstrated therapeutic efficacy of stem cells in the subacute phase.[3] Intravenous (IV) or intra-arterial administration is preferred in the subacute phase,[3] and the lack of efficacy in previous trials may be explained by the low homing rate of cells to the stroke-affected, post-infarct region and their rapid disappearance after being trapped in the lung.[3,4] Importantly, they mainly act through a bystander effect rather than replenishing lost neural cells.[3]

Multilineage-differentiating stress-enduring (Muse) cells are endogenous pluripotent stem cells distributed in the BM, blood, and organ connective tissues as cells positive for the surface marker of pluripotency, stage-specific embryonic antigen-3.[5,6] Because Muse cells are endogenous, there are few safety concerns with their therapeutic use. Because Muse cells detect sphingosine-1-phosphate, an injury signal produced by phosphorylation of membrane-bound sphingosine by damaged cells, they can be delivered intravenously and will selectively migrate to the damage site instead of being trapped in the lung.[7] Within injured tissue, Muse cells replenish damaged/lost cells by spontaneously differentiating into the appropriate cell types and integrating into the correct position to maintain tissue function; thus, they do not require gene introduction or cytokine treatment to be rendered pluripotent and to induce differentiation.[6–10] Furthermore, allogenic Muse cells can be used without human leukocyte antigen (HLA) matching or immunosuppressants, partly due to HLA-G expression, implicated in immunotolerance of immune-privileged tissues like placenta.[7] Finally, donor-derived Muse cells remain incorporated as functional cells in host tissue for an extended time after IV injection without immunorejection.[7]

In rodent ischemic stroke models, human Muse cells differentiated spontaneously into neuronal and glial cells after engraftment into the peri-infarct area.[9,10] Muse cell–derived neurites were incorporated into the pyramidal tract, including the pyramidal decussation, and into the sensory tract, as demonstrated by somatosensory-evoked potentials and formation of synapses with host neurons. These actions led to statistically significant, meaningful functional recovery with no adverse effects for 2–3 months.[9,10]

The proliferation speed of Muse cells is comparable to fibroblasts, and their production can be expanded to clinical scale.[5] Recently, a clinical-grade allogenic Muse cell–based product, CL2020, was developed for IV administration. In an immunodeficient mouse stroke model, CL2020 administered intravenously during the subacute phase migrated to the infarct within 1 day, spontaneously differentiated into neural and glial cells, and improved forelimb motor function compared with vehicle for up to 22 weeks.[11] When engrafted CL2020 cells were ablated by human-selective diphtheria toxin, functional recovery was abrogated, suggesting that the behavioral outcome was due to reconstruction of neuronal circuits by integrated CL2020 cells.[11] Thus, specific homing to the post-infarct region after systemic administration and long-lasting structural regeneration by replenishment of lost cells and long-lasting reconstruction of neural circuits, distinct from a bystander effect, are keys to Muse cell therapeutic outcomes.

In Japan, there are standard treatments for the acute phase of ischemic stroke up to about 2 weeks after onset; however, treatments after the acute phase are limited to rehabilitation and management of potential risk factors for recurrence.[12] Given the efficacy of CL2020 in the regeneration of damaged neuronal networks in a mouse model of stroke,[11] we hypothesized that CL2020 may also be effective in patients in the subacute phase after an ischemic stroke. In agreement with the Japanese Pharmaceuticals and Medical Devices Agency (PMDA), this randomized, double-blind, placebo-controlled trial was an exploratory study of the safety and efficacy of a single IV injection of allogenic CL2020 without HLA matching or immunosuppressants in patients with subacute ischemic stroke who have moderate to severe neurological symptoms and physical dysfunction.

Material and methods

Standard protocol approvals, registrations, and patient consents

This single-center (Tohoku University Hospital, Sendai, Japan), phase 2, randomized, double-blind, placebo-controlled trial (JAPIC Clinical Trials Information site; JapicCTI-184103) was approved by the Tohoku University Hospital Clinical Trial Review Committee (approval number 187001) and conducted in compliance with the Japanese "Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices" and the "Ordinance on Good Clinical Practice of Regenerative Medical Products, etc", and the Declaration of Helsinki. Patients (or family member, if required) provided written informed consent. Patients were enrolled between October 19, 2018 and December 18, 2019.

Participants

Patients (20 to <80 years) who were diagnosed with ischemic stroke, who had apparent motor impairment,

National Institutes of Health Stroke Scale (NIHSS) score ≥6 and modified Rankin Scale (mRS) score ≥3 at enrollment, an mRS score of 0 or 1 before ischemic stroke (patient/family-reported), and who could receive study treatment 14–28 days after stroke onset were eligible. mRS is a scale to evaluate the severity of diseases that cause abnormality in neuromotor function such as cerebrovascular disorders such as cerebral haemorrhage and cerebral infarction and neurological diseases such as Parkinson's disease. Patients' conditions can be classified into 7 stages from "not at all symptomatic" to "death" Change in the rating of this scale is used as a measure of treatment effectiveness. NIHSS is a scale that assesses the neurological severity of stroke, including cerebral infarction, cerebral hemorrhage, and subarachnoid hemorrhage. A total of 11 items including consciousness, motor, sensation and speech are evaluated and scored according to the evaluation table. When all assessments are completed, add up the respective scores. The NIHSS considers 0 to be normal with neurologic severity approaching the maximum 40. Exclusion criteria included the following: decreased consciousness (NIHSS 1 A: 3); clinically important hemorrhagic transformation by computed tomography or magnetic resonance imaging; cognitive impairment (eg, dementia, Alzheimer's disease, Parkinson's disease) that might interfere with evaluation; history of thromboembolism (excluding previous stroke); systolic blood pressure >200 mm Hg or diastolic blood pressure >120 mm Hg; current or previous malignancy; collagen disease or related diseases; diabetes with glycated hemoglobin A1c >10%; active infections requiring treatment; severe complications; current use of systemic steroids or immunosuppressants; positive for hepatitis B virus surface antigen, hepatitis C virus antibody, human immunodeficiency virus antibody, human T-cell leukemia virus type 1 antibody, or syphilis; pregnant or breastfeeding; or a history of hypersensitivity to aminoglycoside antibiotics, human serum albumin preparations, or proteins of bovine or porcine origin. Patients were randomized (2.5:1), stratified by baseline severity (mRS 3 versus 4/5), to receive CL2020 or indistinguishable placebo. An interactive web response system was used for sequence allocation by the minimization method. Patients, investigators, and the sponsor were blinded to allocation. Personnel involved in the preparation of CL2020 or placebo were unblinded but remained separate from other study-related procedures.

Procedures

Clinical-grade CL2020 (1.5×10^7 cells/15 mL) was manufactured from human (allogenic) MSC by Life Science Institute, Inc. (Tokyo, Japan) and frozen until use. The dose was determined by extrapolating the results from a dose-finding study (unpublished) in a mouse model of myocardial infarction to human weight. For each patient, a 15-mL preparation of CL2020 or placebo (the same composition as CL2020 except for the cells) was thawed, diluted in ~37 mL of acetate Ringer's solution, and administered intravenously over 10–15 minutes. All patients followed a standard rehabilitation program. Treatment for stroke, adverse events (AEs), or complications was permitted. Patients were hospitalized at the time of informed consent (≤1 week before randomization). At the investigator's discretion, patients could be discharged any time between 2 and 12 weeks after study treatment but returned to the hospital for scheduled study visits.

Outcomes

The primary endpoint was to evaluate safety for 12 weeks after administration, although safety was assessed for the full 52-week study. Safety measures included type, incidence, and severity of AEs and adverse reactions (ARs; AEs for which a causal relationship with study treatment could not be ruled out).

The key secondary efficacy endpoint was response rate, defined as the percentage of patients who achieved an mRS score of ≤2 at week 12 (responder analysis). Other secondary efficacy endpoints included the following: percentage of patients with mRS score of ≤1; percentage with mRS score of ≤2; improvement in mRS score by ≥2 from baseline; percentage with NIHSS score of ≤1; and changes from baseline in mRS, NIHSS, Stroke Impairment Assessment Set (SIAS), Barthel Index (BI), Fugl-Meyer Motor Scale (FMMS; upper limbs, lower limbs, total), and general health-related quality of life, measured by the patient-reported EuroQol 5-Dimension 5-Level (EQ-5D-5L) index and visual analog scale (VAS).[13,14]

Statistical analysis

The target sample size was based on the key efficacy endpoint (response rate). The probability of having mRS ≤2 at 12 weeks after onset of ischemic stroke is related to NIHSS score at discharge.[15] Using registry data from 88 Japanese patients (age 20–80 years and NIHSS scores of 6–20 at discharge),[16] we estimated a mean predicted responder probability with standard therapy as 8.7%, which was set as the threshold response rate. An expected response rate of 30% for the CL2020 group was considered clinically meaningful by medical experts. As agreed with the PMDA, we could conclude that CL2020 was efficacious if the lower limit of the Clopper-Pearson 95% confidence interval (CI) of the CL2020 response rate was >8.7%. Under these assumptions, 23 patients in the

CL2020 group would provide power >70%; therefore, we aimed to enroll 25 patients in the CL2020 group, assuming a 10% dropout rate. For the placebo group, we aimed to enroll 10 patients, that could be included during the limited recruitment period of the trial.

The lower limit of the 95% CI of the percentage of patients with mRS $\leq$2 at week 12 (response rate) in the CL2020 group was compared with the preset efficacy threshold (8.7% from registry data). Between-group differences in the response rate and other secondary efficacy endpoints were compared using Fisher's exact test (mid-*P* value for responder analysis). Response rates were also determined for subgroups based on baseline characteristics. The distribution in mRS scores was compared between groups using a Wilcoxon rank sum test. Adjusted mean changes from baseline (95% CI) for all efficacy outcomes were calculated using a linear mixed-effects model with fixed effects for group, time point, the interaction of group and time point, and baseline value of the objective variable. In addition to the planned statistical analyses, we conducted Shift analysis as exploratory *post-hoc* test. Shift analysis was performed with ordinal logistic regression using a cumulative logistic model. The group information and the baseline value of mRS are used as explanatory variables, using the clm function in the R language ordinal library. SAS® (SAS Institute, Cary, NC, USA) version 9.3 or higher was used. Statistical significance level was set at 5% (2-sided). The study protocol and statistical analysis plan are available in Supplementary Clinical Study Protocol and Supplementary Statistical Analysis Plan, respectively.

Data availability

To ensure patient privacy is maintained, patient-level data will not be available due to small, single-center study.

Results

Of 43 patients enrolled, 37 were randomized to CL2020 (n = 27) or placebo (n = 10) (Figure 1). Two patients allocated to CL2020 did not receive study treatment because of complications that were protocol deviations. One patient received CL2020 on day 29 after stroke (1 day outside the protocol-specified time frame) but was considered eligible for assessment. Consequently, 25 patients in the CL2020 group and 10 patients in the placebo group received study treatment. All patients completed to week 12; 33 patients completed to week 52. Four additional patients in the CL2020 group and 1 additional patient in the placebo group were excluded from week 28 and week 52 efficacy evaluations because of serious AEs or COVID-19–related difficulties attending the clinic. Except for mRS, which included the patient who died, there were 21 patients in the CL2020 group and 8 patients in the placebo group for week 52 efficacy measures (Supplementary Table 1). Patients who could not attend the clinic were assessed via telephone.

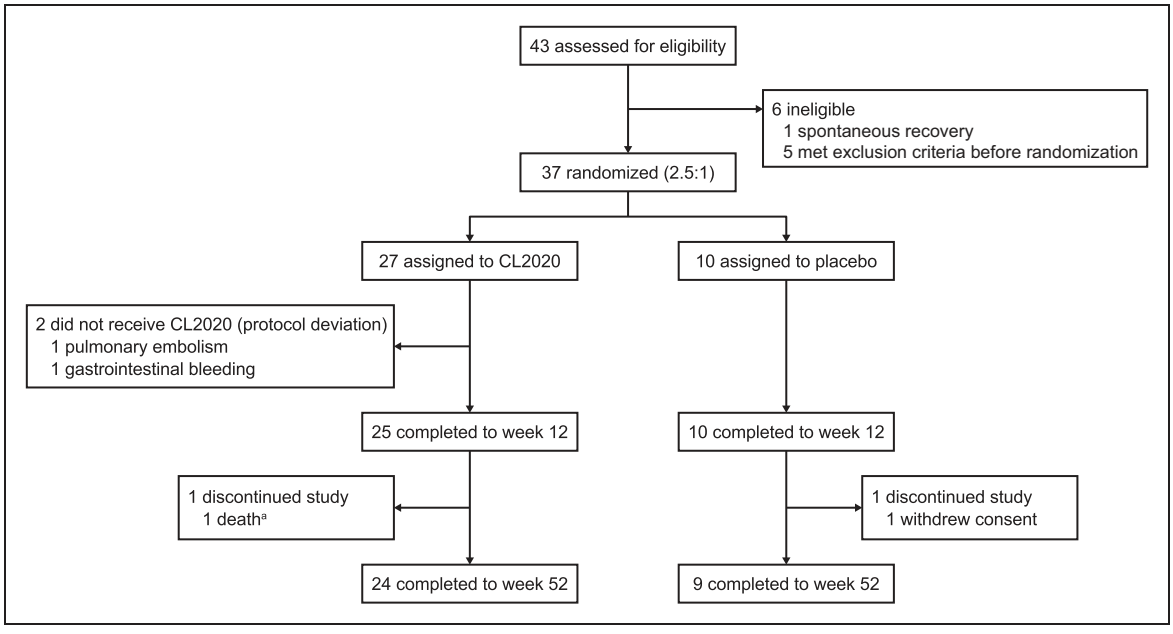


Figure 1. Trial profile.
[a]Due to pneumonia aspiration 345 days after receiving CL2020 (not considered related to study treatment).

Demographics and baseline characteristics were similar in the 2 groups, with some minor imbalances likely due to chance (Table 1; Supplementary Table 2). One patient in the CL2020 group had baseline mRS 3; all other patients had mRS 4 or 5. Baseline NIHSS scores were numerically higher in the placebo group than in the CL2020 group. FMMS total score ($P = 0.041$) and EQ-5D-5L score ($P = 0.026$) was significantly higher in the CL2020 group than in the placebo group.

During the first 12 weeks after administration, AEs were experienced by 24 (96%) and 10 (100%) patients in the CL2020 and placebo groups, respectively, most commonly gastrointestinal disorders, including diarrhea (CL2020, 28%; placebo, 30%) and constipation (CL2020, 24%; placebo, 60%) (Table 2). The occurrence of psychiatric disorders (CL2020, 16%; placebo, 70%; $P = 0.004$) and insomnia (CL2020, 16%; placebo, 60%; $P = 0.016$) were significantly low in the CL2020 group compared with the placebo group. Up to week 12, ARs occurred in 7 (28%) patients in the CL2020 group and 1 (10%) patient in the placebo group. In the CL2020 group, 3 (12%) patients had hair discoloration from gray/white to black by week 12, and 3 additional patients had hair discoloration between week 12 and week 52, for a total of 6 patients (24%); no patients in the placebo group had this AR.

Table 1. Patient demographics and baseline characteristics.

Variable	CL2020 (N = 25)	Placebo (N = 10)	*P* value[a]
Age, y	64.0 ± 12.1	59.2 ± 11.0	0.159
<65	8 (32)	5 (50)	0.326
≥65	17 (68)	5 (50)	
Sex			
Male	20 (80)	6 (60)	0.393
Female	5 (20)	4 (40)	
Days from onset of stroke to study treatment administration	19.9 ± 4.5	20.3 ± 4.8	0.826
<21 d	15 (60)	6 (60)	1.000
≥21 d	10 (40)	4 (40)	
Previous treatment (from onset of stroke to informed consent)[b]	25 (100)	10 (100)	
Reperfusion therapy	10 (40)	5 (50)	0.712
Alteplase	7 (28)	4 (40)	0.689
Thrombectomy	7 (28)	4 (40)	0.689
Stroke type classification			
Cardioembolic stroke	1 (4)	2 (20)	0.190
Atherothrombotic stroke	10 (40)	3 (30)	0.709
Lacunar stroke	3 (12)	1 (10)	1.000
Unknown	5 (20)	3 (30)	0.661
Other	6 (24)	1 (10)	0.644
mRS	4.1 ± 0.4	4.4 ± 0.5	0.057
3	1 (4)	0	0.527
4 or 5	24 (96)	10 (100)	
NIHSS	9.8 ± 3.5	14.1 ± 6.2	0.051
<10	15 (60)	4 (40)	0.290
≥10	10 (40)	6 (60)	
SIAS	41.4 ± 13.2	32.6 ± 11.0	0.062
BI	32.0 ± 26.2	23.5 ± 19.7	0.399
FMMS			
Upper limbs	18.8 ± 18.8	4.8 ± 3.5	0.064
Lower limbs	13.3 ± 10.1	6.7 ± 6.4	0.073
Total	32.1 ± 26.6	11.5 ± 9.5	0.041*
EQ-5D-5L			
Index	0.4349 ± 0.1670	0.2385 ± 0.1898	0.026*
VAS	47.7 ± 27.6	30.0 ± 22.0	0.104

BI: Barthel Index; EQ-5D-5L: EuroQol 5-Dimension 5-Level; FMMS: Fugl-Meyer Motor Scale; mRS: modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale; SIAS: Stroke Impairment Assessment Set; VAS: visual analog scale.

Data are mean ± SD or n (%).

[a]Wilcoxon rank sum test for age, mRS, NIHSS, and days from onset of stroke to study treatment administration, and Fisher's exact test for others.

[b]Patients may be counted in more than 1 category.

*$P \leq 0.05$.

Table 2. Safety overview, most common adverse events, and adverse reactions to week 12 and to week 52.

Variable	To week 12			To week 52		
	CL2020 (N = 25)	Placebo (N = 10)	*P* value[a]	CL2020 (N = 25)	Placebo (N = 10)	*P* value[a]
Adverse events						
Any AE	24 (96)	10 (100)	1.000	25 (100)	10 (100)	1.000
AEs leading to discontinuation or death	0	0	1.000	1 (4)	0	1.000
Serious AEs	1 (4)	0	1.000	5 (20)	1 (10)	0.649
AEs occurring in ≥2 patients by week 52 in the CL2020 group by SOC and PT[b]						
Gastrointestinal disorders	15 (60)	7 (70)	0.709	16 (64)	7 (70)	1.000
Diarrhea	7 (28)	3 (30)	1.000	7 (28)	3 (30)	1.000
Constipation	6 (24)	6 (60)	0.059	7 (28)	6 (60)	0.123
Vomiting	2 (8)	1 (10)	1.000	2 (8)	1 (10)	1.000
Abdominal pain	2 (8)	0	1.000	2 (8)	0	1.000
Toothache	1 (4)	0	1.000	2 (8)	0	1.000
Investigations	2 (8)	1 (10)	1.000	5 (20)	1 (10)	0.649
Weight increased	0	0	1.000	2 (8)	0	1.000
Infections and infestations	7 (28)	2 (20)	1.000	11 (44)	2 (20)	0.259
Urinary tract infection	4 (16)	0	0.303	4 (16)	0	0.303
Nasopharyngitis	0	1 (10)	0.286	3 (12)	1 (10)	1.000
Paronychia	1 (4)	0	1.000	2 (8)	0	1.000
Metabolism and nutrition disorders	4 (16)	0	0.303	8 (32)	0	0.073
Hypoglycemia	2 (8)	0	1.000	2 (8)	0	1.000
Diabetes mellitus	0	0	1.000	4 (16)	0	0.303
Musculoskeletal and connective tissue disorders	9 (36)	4 (40)	1.000	11 (44)	4 (40)	1.000
Musculoskeletal pain	4 (16)	1 (10)	1.000	6 (24)	1 (10)	0.644
Pain in extremity	4 (16)	1 (10)	1.000	4 (16)	1 (10)	1.000
Nervous system disorders	6 (24)	3 (30)	0.694	9 (36)	3 (30)	1.000
Cerebral infarction	2 (8)	1 (10)	1.000	3 (12)	1 (10)	1.000
Headache	2 (8)	1 (10)	1.000	3 (12)	1 (10)	1.000
Psychiatric disorders	4 (16)	7 (70)	0.004**	5 (20)	8 (80)	0.002**
Insomnia	4 (16)	6 (60)	0.016*	4 (16)	7 (70)	0.004**
Renal and urinary disorders	3 (12)	1 (10)	1.000	4 (16)	2 (20)	1.000
Hematuria	2 (8)	0	1.000	3 (12)	0	0.542
Skin and subcutaneous tissue disorders	8 (32)	4 (40)	0.706	13 (52)	5 (50)	1.000
Hair colour changes	3 (12)	0	0.542	6 (24)	0	0.152
Drug eruption	2 (8)	0	1.000	3 (12)	0	0.542
Hemorrhage subcutaneous	2 (8)	0	1.000	2 (8)	0	1.000
Pruritus	0	1 (10)	0.286	2 (8)	2 (20)	0.561
Rash	1 (4)	1 (10)	0.496	2 (8)	1 (10)	1.000
Adverse reactions						
Any AR	7 (28)	1 (10)	0.390	10 (40)	1 (10)	0.120
ARs leading to discontinuation or death	0	0	1.000	0	0	1.000
Serious ARs	1 (4)	0	1.000	1 (4)	0	1.000
ARs by SOC and PT						
Gastrointestinal disorders	1 (4)	0	1.000	1 (4)	0	1.000
Abdominal pain	1 (4)	0	1.000	1 (4)	0	1.000
Vomiting	1 (4)	0	1.000	1 (4)	0	1.000
Hepatobiliary disorders	0	1 (10)	0.286	0	1 (10)	0.286
Hepatic function abnormal	0	1 (10)	0.286	0	1 (10)	0.286
Metabolism and nutrition disorders	2 (8)	0	1.000	2 (8)	0	1.000
Hyperuricemia	1 (4)	0	1.000	1 (4)	0	1.000
Dyslipidemia	1 (4)	0	1.000	1 (4)	0	1.000
Nervous system disorders	1 (4)	0	1.000	1 (4)	0	1.000
Headache	1 (4)	0	1.000	1 (4)	0	1.000

(continued)

Table 2. Continued.

Variable	To week 12			To week 52		
	CL2020 (N = 25)	Placebo (N = 10)	*P* value[a]	CL2020 (N = 25)	Placebo (N = 10)	*P* value[a]
Status epilepticus	1 (4)	0	1.000	1 (4)	0	1.000
Skin and subcutaneous tissue disorders	3 (12)	0	0.542	6 (24)	0	0.152
Hair colour changes	3 (12)	0	0.542	6 (24)	0	0.152
Hair growth abnormal	0	0	1.000	1 (4)	0	1.000
Hypertrichosis	0	0	1.000	1 (4)	0	1.000
Skin discoloration	0	0	1.000	1 (4)	0	1.000

AE: adverse event; AR: adverse reaction; PT: preferred term; SOC: system organ class.
Data are n (%).
[a]Fisher's exact test.
[b]Japanese Medical Dictionary for Regulatory Activities, version 23.0.
*$P \leq 0.05$, **$P \leq 0.01$.

A 56-year-old man had a serious AR of Grade 4 status epilepticus 86 days after CL2020 administration, which was treated with antiepileptic drugs. The patient recovered at day 93 but experienced 2 additional serious AEs that were considered sequelae of status epilepticus (epileptic encephalopathy on day 94; esophageal ulcer due to antiplatelet drugs on day 129). At study end, his symptoms were stable.

During the full 52 weeks, all patients had at least 1 AE (Table 2, Supplementary Table 3). Seven serious AEs occurred in 5 patients in the CL2020 group (status epilepticus [described above], epileptic encephalopathy, femur fracture, pneumonia aspiration, coronary artery stenosis, sepsis, esophageal ulcer) and 1 patient in the placebo group (epilepsy). Non-serious ARs occurred in 3 additional patients in the CL2020 group between week 12 and week 52 (total of 10 [40%]); no ARs occurred in the placebo group after week 12.

In the responder analysis, 10 (40%) patients in the CL2020 group had mRS 0–2 at week 12 compared with 1 (10%) patient in the placebo group (Figure 2). The lower limit of the 95% CI for the CL2020 response rate was 21.1%, far exceeding the assumed efficacy threshold of 8.7%. The estimated difference between groups in response rate at week 12 was 30.0% (95% CI, –7.8 to 65.2, mid-$P = 0.080$) and remained at ~30% at week 52 (Supplementary Table 4). In subgroup analyses, there was no remarkable effect of sex, baseline mRS, baseline NIHSS, days from stroke onset to study treatment, presence/absence of reperfusion therapy, or stroke type on the response rate (Supplementary Figure 1).

The adjusted mean change from baseline in mRS score was numerically greater in the CL2020 group than in the placebo group after week 4 ($P = 0.079$ at week 12) (Figure 3(a); Supplementary Table 5). The difference between groups was largest between

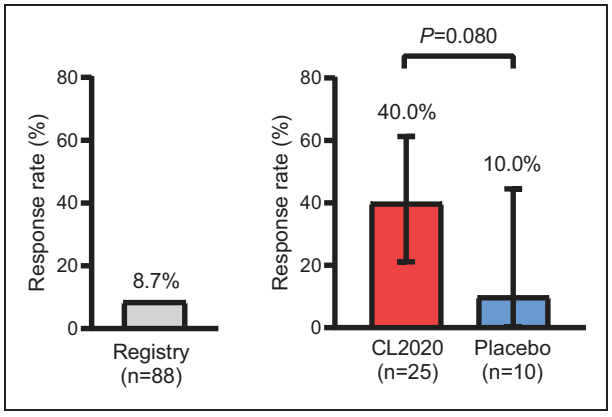


Figure 2. Responder analysis of patients with mRS scores of 0–2 at Week 12.
Shown are mean (95% CI) response rates in the CL2020 (40.0% [21.1–61.3]) and placebo (10.0% [0.3–44.5]) groups; the threshold response rate of 8.7% based on registry data[16] is also shown. The lower limit of the 95% CI in the CL2020 group was higher than the 8.7% seen in the registry data. The difference between the CL2020 and placebo groups was analyzed by Fisher's exact test (mid-*P* value). mRS = modified Rankin Scale.

weeks 12 and 28 (Figure 3(b)). Across 52 weeks, the distribution of mRS scores in the CL2020 group shifted to mRS 1 or 2 (15/22 patients; 68% at week 52); notably, 2 (8%) patients achieved mRS 1 at week 12 and 7 (32%) patients at week 52 (Figure 3(c); Supplementary Table 6). No patients in the placebo group achieved mRS 1. The distribution of mRS scores was significantly different between groups at weeks 8 ($P = 0.029$) and 12 ($P = 0.034$) (Supplementary Table 6). The percentage of patients with mRS ≤1 was greater in the CL2020 group compared with the placebo group and stayed at ~30% between weeks 28 and 52, although differences were not statistically significant (Supplementary Table 7). The percentage of patients whose mRS

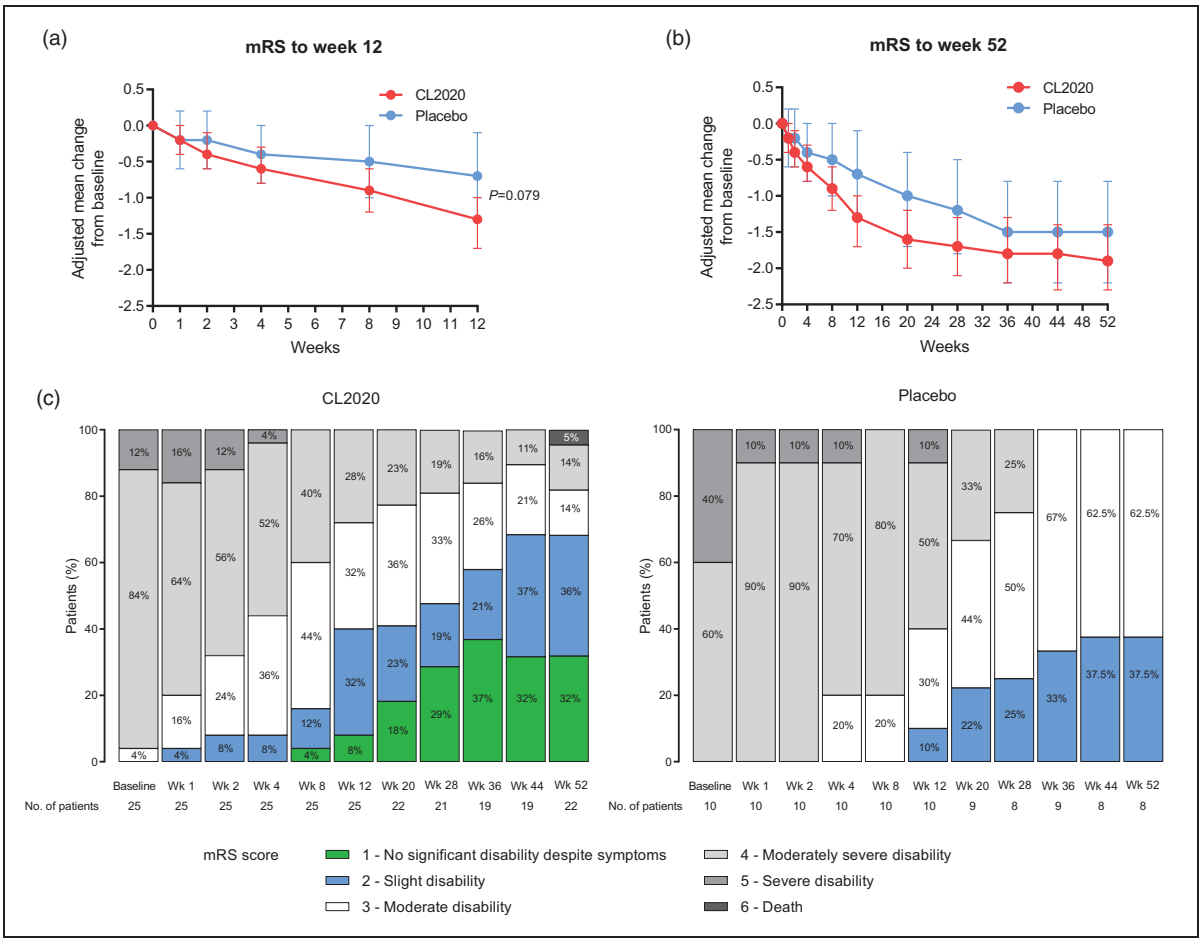


Figure 3. Adjusted Mean (95% CI) change from baseline to (a) Week 12 and (b) Week 52 in mRS scores in the CL2020 and placebo groups; (c) Distribution of mRS scores in the CL2020 and placebo groups from baseline to Week 52.
mRS: modified Rankin Scale; Wk: week.

scores improved by ≥2 points was greater in the CL2020 group than in the placebo group, with the greatest difference at week 12 (Supplementary Table 8); between-group differences were not statistically significant. Shift analysis demonstrated that 12-week mRS tended towards lower in the CL2020 group, but the difference was not significant (common odds ratio of a shift in score on the mRS in the CL2020 group, 3.69; 95%CI, 0.83 – 16.47; $P = 0.087$).

Improvements in FMMS upper limb and total scores, but not lower limb scores, were greater in the CL2020 group compared with the placebo group and were statistically significant after week 4 (Figure 4; Supplementary Table 5 and Supplementary Figure 2). At week 52, 5/21 patients (23.8%) in the CL2020 group had NIHSS score ≤1, compared with 0/8 patients in the placebo group (Supplementary Table 9). Improvements in SIAS scores were numerically greater in the CL2020 group than in the placebo group. There were no apparent differences between groups in the change from baseline for NIHSS or BI. Changes from baseline in EQ-5D-5L scores were generally greater in the CL2020 than in the placebo group (significant at week 12; Supplementary Table 5 and Supplementary Figure 2).

Classification of evidence

This study provides Class I evidence that CL2020 improved functional outcomes in patients with subacute ischemic stroke with no major safety issues.

Discussion

This randomized, double-blind, placebo-controlled study evaluated the safety and efficacy of allogenic cells for treating patients with ischemic stroke–induced neurological deficits and physical dysfunction during the subacute phase. There were no major safety issues associated with a single IV injection of CL2020, although 1 patient experienced status epilepticus. In the key responder analysis, the lower 95% CI in the

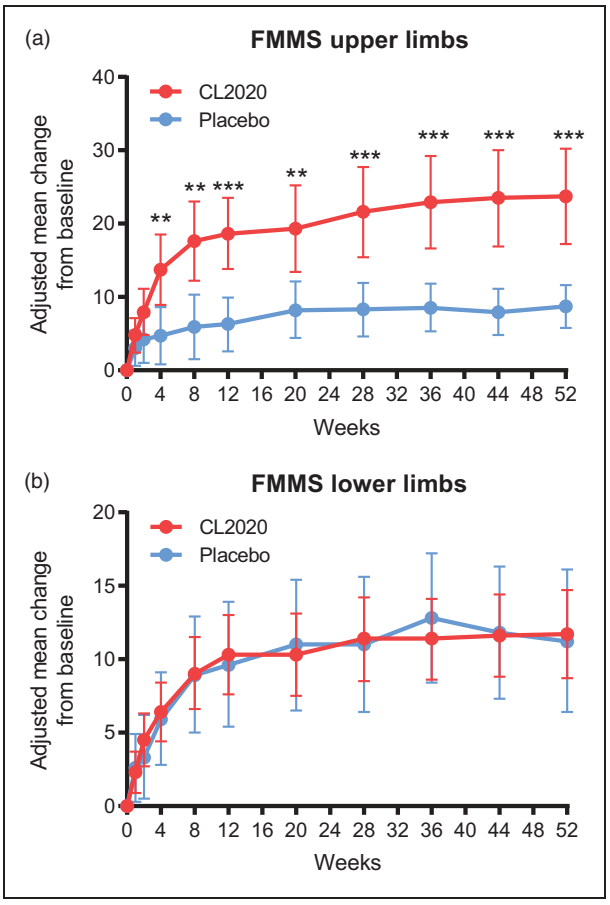


Figure 4. Adjusted mean (95% CI) change from baseline in (a) FMMS upper limb score and (b) FMMS lower limb score in the CL2020 (N = 19–25) and placebo (N = 8–10) groups. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ between groups. FMMS: Fugl-Meyer Motor Scale.

CL2020 group exceeded the threshold response rate of 8.7%, which was based on Japanese patient registry data[16] and supported by international data.[17] Further, the percentage of patients with mRS ≤2 at week 12 was higher in the CL2020 group than in the placebo group (40% vs 10%). Both the change from baseline and the distribution of mRS scores supported the efficacy of CL2020 for improving functional recovery for up to 52 weeks. In addition, the CL2020 group exhibited statistically significant improvements in FMMS upper limb and total scores. Symptoms continued to improve during the first 6 months, and numerical differences between CL2020 and placebo were maintained between 6 and 12 months. Taken together, these promising results suggest that CL2020 may be a possible treatment for subacute stroke.

To date, 6 randomized studies in the subacute phase have been conducted, all with IV infusion of autologous cells,[18–23] but only 2 reported positive results in functional outcomes.[18,22] In an open-label study with 2 injections of autologous MSCs (MSC n = 5; control n = 25), there was a significant improvement of BI at 3 and 6 months, and a tendency for mRS improvement over 12 months that was not statistically significant.[18] Another open-label, observer-blinded study with 2 injections of autologous MSCs (MSC n = 16; control n = 36) showed a significant increase in the proportion of patients with mRS 0–3 in the MSC group at a median of 3.2 years' follow-up.[22] In the current study, in addition to a greater proportion of patients achieving mRS ≤2 at week 12, 7 patients in the CL2020 group—but none in the placebo group—achieved mRS 1 (ie, no significant disability), which is rare for patients with an initial mRS 4 or 5 in the subacute phase. By comparison, the percentage of patients achieving mRS 0 or 1 was 2.6% at 90 days with MSCs,[19] 2% at 180 days with BM-MNCs,[23] and 6.3% at median follow-up of 3.2 years with MSCs,[22] all with IV autologous cells. Although differences in the use of allogenic/autologous cells, number of injections, and blinding methods make comparison difficult, the percentage of CL2020-treated patients with mRS 1 was 8%, 29%, and 32% at weeks 12, 28, and 52, respectively. Allogenic CL2020 has several advantages over autologous cells: invasive treatments such as BM aspiration can be avoided, and Muse cells are ready for administration without culturing because they are already enriched.

Although improvements in secondary efficacy endpoints, such as SIAS, BI, and the percentage of patients with NIHSS score of ≤1, were not greater with CL2020 compared with placebo, significantly greater improvements in FMMS upper limb and total scores were observed from week 4 to week 52. Such early improvements in upper limb function are difficult to achieve and are directly related to improving mRS to 0 or 1. Previous research suggests that upper limb function is particularly important to "dressing" (changing clothes) as an activity of daily living and is highly correlated with patients becoming independent.[24] Conversely, lower limb function improved equally well in both the CL2020 and placebo groups. This may be related to general effects of rehabilitation therapy and/or compensation from the unaffected side of the body. The lack of effect on NIHSS scores in patients with subacute stroke is not surprising given that the scale primarily measures stroke severity in the acute phase. The BI measures activities of daily living, and improvements may primarily reflect rehabilitation therapy; this could account for the similar improvement in both groups. Moreover, differences in BI between groups may not be detected due to ceiling effects.[25] Quality of life, measured by EQ-5D-5L index and VAS scores, was significantly greater in the CL2020 group than in the placebo group at week 12.

Similar to the BI, it is possible that rehabilitation therapy contributed to improvements in quality of life in both groups over time.

As in previous stem cell studies,[4] no major safety issues related to CL2020 were observed. Most AEs occurred at similar frequencies in both groups, although treatment-related hair discoloration from gray/white to black was observed in 6 patients in the CL2020 group. Notably, because CL2020 is an enriched Muse cell preparation, the number of cells injected is around 4–20 times lower than used in other cell-based systemic therapies,[18–23] thereby lowering the risk of pulmonary embolism.[26] Although there was 1 serious AR of status epilepticus in a CL2020-treated patient, epilepsy is a known complication of stroke, reported in approximately 6%–7% of patients,[27,28] with an incidence of 2.9% during the first 6 months after stroke.[27] Thus, the single status epilepticus event was not considered unusual, although its relationship to CL2020 could not be ruled out. Given that seizures in a small percentage of patients occurred in other stem cell studies,[29,30] further research is required to confirm whether these treatments increase the frequency of post-stroke epilepsy.

The main limitation of this exploratory study was that it was conducted at a single medical center, and although the sample size was agreed with the regulatory authority, the PMDA, a larger study conducted at multiple centers is required to confirm the results. We are currently planning a larger phase 3 study, which we expect will also address the slight imbalance in baseline NIHSS scores seen in this phase 2 study.

In conclusion, there is a large unmet medical need for effective, long-lasting treatments for subacute ischemic stroke. This exploratory study suggests that a single IV injection of the clinical-grade allogenic Muse cell product CL2020, without immunosuppressants or HLA matching, may be a safe and effective treatment for subacute stroke, with the potential to make regenerative medicine more accessible.

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Authors' contributions

All authors participated in the interpretation of study results, and in the drafting, critical revision, and approval of the final version of the manuscript. K.N., H.E., S.I., A.H., M.I., and T.T. were involved in the study design. K.N., S.O., H.E., and S.I. were study investigators. K.N., S.O., and K.A. contributed to data collection. A.H., M.I., and T.T. conducted the statistical analyses. All authors had access to the data table listings (prepared by Life Science Institute, Inc.), which were used to prepare the Results and Discussion sections of this report. All authors had full access to all the data in this study and take final responsibility for the decision to submit this report for publication.

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