Circulation Journal
Circ J 2020; 84: 1189–1192
doi:10.1253/circj.CJ-20-0307

RAPID COMMUNICATION

Safety and Efficacy of Human Muse Cell-Based Product for Acute Myocardial Infarction in a First-in-Human Trial

Toshiyuki Noda, MD, PhD; Kazuhiko Nishigaki, MD, PhD; Shinya Minatoguchi, MD, PhD

Background: Because ST-elevation myocardial infarction (STEMI) extensively damages the heart, regenerative therapy with pluripotent stem cells such as multilineage-differentiating stress enduring (Muse) cells is required.

Methods and Results: In a first-in-human study, 3 STEMI patients with a left ventricular ejection fraction (LVEF) ≤45% after successful percutaneous coronary intervention received intravenously 1.5×10^7 cells of a human Muse cell-based product, CL2020. The safety and efficacy on LVEF and wall motion score index (WMSI) were evaluated for 12 weeks. No adverse drug reaction was noted. LVEF and WMSI were markedly improved.

Conclusions: The first-in-human intravenous administration of CL2020 was safe and markedly improved LV function in STEMI patients.

Key Words: Acute myocardial infarction; Muse cell-based product; Safety

Acute myocardial infarction (AMI) is one of the leading causes of morbidity and mortality worldwide despite the prevalence of percutaneous coronary intervention (PCI).[1] AMI often extensively damages the heart, leading to heart failure because of left ventricular (LV) dysfunction and remodeling. Stem or progenitor cell therapy is a potential treatment to replenish cardiomyocytes, but many clinical trials using bone marrow (BM)-mesenchymal stem cells and BM-mononucleated cells have demonstrated no or minimal efficacy in improving cardiac function.[2] Therefore, an alternative cell source with potent reparative and regenerative abilities is required. Multilineage-differentiating stress enduring (Muse) cells are pluripotent stem cells with positive stage-specific embryonic antigen-3; they express pluripotency markers such as Sox2, Oct3/4, and Nanog, and differentiate into cells of all 3 germ layers from a single cell.[3–6]

We recently reported that intravenous injection of BM-derived Muse cells markedly reduced the myocardial infarct size, improved LV function, and attenuated LV remodeling through regeneration of working cardiomyocytes and vessels and paracrine effects in a rabbit model of AMI.[7] After completion of the necessary process, we performed a first-in-human clinical trial using an allogenic human Muse cell-based product, CL2020, for the treatment of patients with STEMI.

Methods

The protocol was approved by the institutional review boards and ethics committees (approval numbers 2-321 and 201811012, respectively). Written informed consent was given by the patients, and the clinical study was conducted in compliance with current Good Clinical Practice standards and according to the Declaration of Helsinki. The trial registration: The Japic CTI (Japan Pharmaceutical Information Center Clinical Trials Information) number is 183834. The clinical trial notification for this study was submitted and the study was allowed to start by Japan Pharmaceuticals and Medical Devices Agency and the Ministry of Health, Labour and Welfare.

Outcome Measures

The primary outcome of the present study was the safety and adverse-event profile of CL2020 for up to 12 weeks. The secondary outcome was the effectiveness of CL2020 on cardiac function.

Study Design

The clinical-grade Muse cell-based product CL2020 (1.5×10^7 cells/15 mL of frozen preparation) was manufactured by Life Science Institute, Inc. (Tokyo, Japan).[8] The process of CL2020 preparation was as follows. For administration, CL2020 (15 mL) was diluted by 37 mL of Ringer's acetate solution, and the total of 52 mL of CL2020 solution was intravenously infused slowly for 10–15 min into STEMI patients with LVEF ≤45%, because LVEF ≤45% in STEMI patients has been reported to be associated with a poorer prognosis.[9] To assess the primary outcome, vital signs were measured, and a medical

Received April 7, 2020; revised manuscript received May 6, 2020; accepted May 25, 2020; J-STAGE Advance Publication released online June 11, 2020 Time for primary review: 15 days

Gifu Prefectural General Medical Center, Gifu (T.N.); Gifu Municipal Hospital, Gifu (K.N., S.M.), and Department of Circulatory and Respiratory Advanced Medicine, Gifu University Graduate School of Medicine, Gifu (S.M.), Japan

S.M. is a principal investigator.

Mailing address: Shinya Minatoguchi, MD, PhD, Gifu Municipal Hospital, 7-1 Kashimacho, Gifu 500-8513, Japan. E-mail: minatos@gifu-u.ac.jp

ISSN-1346-9843 All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

Table 1. Patients' Background Characteristics

Characteristic	(n=3)
Age, years	
Mean	64.3±0.6
Male, n (%)	3 (100.0)
Hypertension, n (%)	3 (100.0)
Hyperlipidemia, n (%)	2 (66.7)
Diabetes, n (%)	3 (100.0)
Smoker (current or former), n (%)	3 (100.0)
Family history of coronary artery disease, n (%)	0 (0.0)
Coronary artery disease, n (%)	
1-vessel	1 (33.3)
2-vessel	1 (33.3)
3-vessel	1 (33.3)
Infarct treatment	
Infarct-related vessel, n (%)	
Left anterior descending coronary artery	2 (66.7)
Left circumflex artery	0 (0.0)
Right coronary artery	1 (33.3)
Time from onset to PCI, min	296.7±49.7
Onset-to-door time, min	229.3±49.3
Door-to-balloon time, min	67.3±32.6
TIMI flow grade after PCI, n (%)	
TIMI flow grade 3	3 (100.0)
Medications, n (%)	
ACEI or ARB	3 (100.0)
β-blocker	3 (100.0)
Calcium-channel blocker	1 (33.3)
Aspirin	3 (100.0)
Antiplatelet	2 (66.7)
Cell therapy	
Time from reperfusion to infusion of study therapy, days	4.1±1.0
No. of cells injected, $\times 10^5$ cells/kg	2.1±0.1

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

examination, infectious disease test, hematologic test, blood biochemical test, urinary test, and an inflammatory cytokine test were consecutively performed before and after the administration of CL2020 for up to 12 weeks. To assess the secondary outcome, LVEF was measured by echocardiography with the modified Simpson's method before and 24 h, 8 days, and 2, 4, 8, and 12 weeks after administration of CL2020. Regional LV function was evaluated by the wall motion score index (WMSI) using a 16-segment model for LV segmentation, as recommended by the American Society for Echocardiography.[10] Single-photon emission computed tomography (SPECT) using Tc-MIBI or 99 mTc-Tetrofosmin was performed before and at 12 weeks after the administration of CL2020. Additional details of patient recruitment, administration criteria, safety assessment, and post CL2020 administration examination are provided in the **Supplementary File**.

Statistical Analysis

The data are shown as the mean±standard deviation (mean±SD). Regarding changes in LVEF and WMSI measured by echocardiography, estimates, confidence intervals (CI), and P-values are based on a mixed model for repeated measures (MMRM) of the change from baseline values, with fixed effects for visits and baseline values (SAS® Proprietary Software Release 9.4 TS1M4).

Results

Patient Enrollment

The clinical study was initiated in January 2018 and 3 patients (**Table 1**) were enrolled for intravenous administration of CL2020 after successful PCI.

Safety and Adverse Events of CL2020

No adverse events related to CL2020 were observed in any of the 3 patients based on results of hematologic and biochemical tests or levels of inflammatory cytokines (**Table 2**). In both the acute and later phases during the 12-week study period, there were neither serious adverse events nor adverse events such as death, fatal arrhythmia, shock, infection or MACE (major adverse cardiovascular event) (**Supplementary Table 1**).

Effectiveness of CL2020 on LVEF and WMSI

LVEF significantly increased from 40.7±1.5 to 43.3±2.1, 47.3±3.2, 49.3±3.2, 47.7±3.2, 50.0±3.0, and 52.0±2.6 before and 24 h, 8 days, and 2, 4, 8, and 12 weeks after the administration of CL2020, respectively (**Figure A**). WMSI significantly decreased from 1.594±0.162 to 1.208±0.072 and 1.188±0.063 before and 8 and 12 weeks after the administration of CL2020, respectively (**Figure B**).

Effect of CL2020 on SPECT

SPECT was performed in 2 patients, excluding 1 patient because of atrial fibrillation. Quantitative measurement of LV function is shown in **Supplementary Table 2**.

Discussion

We conducted the first-in-human transplantation of an allogenic Muse cell-based product, CL2020, for the treatment of AMI in 3 patients with STEMI. No adverse events related to CL2020 were observed for up to 12 weeks after administration. LVEF as assessed by echocardiography markedly improved from 40.7±1.5% to 52.0±2.6% at 12 weeks.

The primary outcome measure was to confirm the safety of CL2020. None of the 3 patients showed any adverse side effects on vital signs, medical examination, infectious disease test, hematologic test, blood biochemical test, urinary test, or inflammatory cytokine test for up to 12 weeks. Moreover, there were no fatal arrhythmias, shock, infections, or thrombotic disorders, which are safety concerns associated with cell therapies. This may not be surprising because CL2020 consists of Muse cells, which reside naturally in human organs such as BM,[4] skin,[5] adipose tissue,[6,11] connective tissue of various organs,[12] and in the peripheral blood.[13,14] The most important finding in the present study was that CL2020 is safe when intravenously administered to STEMI patients. As for tumorigenicity, because the study only covered the 12 weeks after administration of CL2020, evaluation of tumorigenicity was not included. A longer period of observation is required to evaluate tumorigenicity, although no tumorigenicity was confirmed during the 6 months after Muse cells were administered in a rabbit

Table 2. Assessment of Safety of CL2020

	Baseline	2 weeks	4 weeks	8 weeks	12 weeks
Hematologic tests					
Hemoglobin (g/dL)	13.87±0.38	13.63±0.40	14.07±0.70	13.90±0.82	14.00±0.96
Red blood cells ($10^4/\mu$L)	453.7±18.6	442.7±8.5	466.7±31.3	460.7±16.9	459.7±33.6
White blood cells (/μL)	7,540.0±2,636.4	7,106.7±1,450.6	8,673.3±1,391.5	7,610.0±1,052.8	6,793.3±1,479.2
Platelets ($10^4/\mu$L)	17.47±3.71	22.27±10.35	18.27±4.81	21.47±5.25	21.23±7.62
Biochemical tests					
Troponin I (pg/mL)	20,753.7±15,453.1	116.3±128.2	–	–	–
Creatine kinase (U/L)	410.0±243.3	68.3±9.1	120.7±5.1	324.7±410.0	175.0±126.0
LDH (U/L)	706.7±355.0	235.0±33.8	281.3±80.4	293.0±105.7	258.7±80.6
ALT (U/L)	40.3±20.5	25.3±14.7	21.0±4.4	28.0±9.0	24.7±8.4
AST (U/L)	71.0±37.0	18.7±5.0	20.0±2.6	29.0±12.2	24.7±8.3
ALP (U/L)	226.0±21.5	303.0±30.6	314.0±29.5	334.7±30.1	335.7±69.7
γ-GTP (U/L)	44.0±30.6	38.7±27.8	36.3±20.2	52.0±23.4	58.3±37.9
Total bilirubin (mg/dL)	0.75±0.14	0.71±0.34	1.04±0.49	0.97±0.41	0.78±0.23
BUN (mg/dL)	13.5±4.3	14.8±5.3	15.2±4.3	15.6±1.9	15.6±1.5
Creatinine (mg/dL)	0.760±0.212	0.850±0.182	0.850±0.227	0.773±0.129	0.793±0.196
Albumin (g/dL)	3.50±0.40	3.80±0.10	4.43±0.51	4.47±0.40	4.33±0.38
CRP (mg/dL)	1.010±0.149	0.663±0.428	0.273±0.176	0.183±0.110	0.113±0.023
Inflammatory cytokines					
IL-1β (pg/mL)	0.1250±0.0000	–	0.1250±0.0000	–	0.1250±0.0000
TNFα (pg/mL)	2.610±1.182	–	2.783±0.387	–	2.297±0.217
IL-6 (pg/mL)	5.263±1.209	–	5.363±4.998	–	1.663±0.420
INFγ (pg/mL)	1.663±0.179	–	1.560±0.000	–	1.560±0.000

Data are shown as the mean±SD (n=3). Regarding inflammatory cytokines, if the measured value was below the low limit of quantification (LLOQ), the value was replaced by the value of LLOQ. LLOQ of each cytokine was: IL-1β: 0.125 pg/mL, TNFα: 0.55 pg/mL, IL-6: 0.300 pg/mL, INFγ: 1.56 pg/mL. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein; GTP, glutamyl transpeptidase; IL, interleukin; INF, interferon; LDH, lactate dehydrogenase; TNF, tumor necrosis factor.

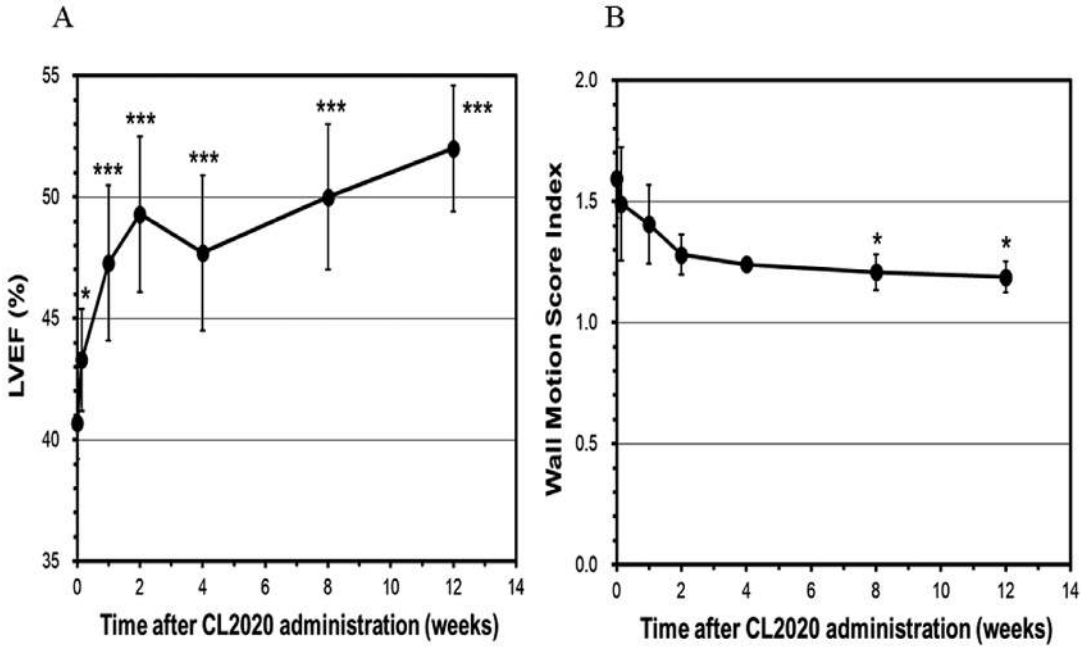


Figure. Changes in cardiac function before and after the administration of CL2020. (**A**) Left ventricular ejection fraction (LVEF) and (**B**) wall motion score index (WMSI) before and 24 h, 8 days, and 2, 4, 8, and 12 weeks after the administration of CL2020. *P<0.05, **P<0.01, ***P<0.001.

model of AMI.[7]

The secondary outcome measure was to assess the effects of CL2020 on LVEF and WMSI. CL2020 gradually but significantly increased LVEF until 12 weeks (**Figure A**), showing a marked increase of 28% at 12 weeks from the baseline value. WMSI significantly decreased at 8 and 12 weeks (**Figure B**), suggesting that regional LV systolic function was significantly improved by CL2020. We recently reported that intravenous administration of 3×10^5 of Muse cells significantly increased LVEF at 2 weeks, 2 months, and even at 6 months (allograft) after Muse cell implantation without immunosuppressant drugs in a rabbit model of AMI.[7] The efficacy of allograft Muse cells suggests immunomodulatory or immunosuppressive effects,[7] which may partially explain the safety and efficacy of CL2020 in the human STEMI patients. We also recently reported that Muse cells are mobilized into the peripheral blood in the acute phase in patients with STEMI, and that patients with a higher number, but not those with a lower number, of mobilized Muse cells showed a significant improvement of LVEF in the chronic phase at 6 months.[14] These findings also suggest the efficacy of CL2020, a Muse cell-based product, in patients with STEMI.

To further evaluate the effectiveness of CL2020, a randomized placebo controlled clinical trial should be performed as the next step and we have scheduled such a trial in the near future.

In conclusion, the intravenous administration of CL2020 did not lead to any adverse side effects and markedly increased LVEF in 3 patients with STEMI. A double-blind placebo-controlled clinical trial is warranted.

Acknowledgment

This study was Funded by Life Science Institute, Inc.

We are grateful to Atsushi Satake, Shinji Yasuda, Hirotaka Murase, Hisaaki Komaki, Shinya Baba, Tomoki Kubota, Shinsuke Ojio (Gifu Municipal Hospital), Masazumi Arai, Shintaro Tanihata, Koji Ono, Makoto Iwama, Shunichiro Warita, Tai Kojima, Takashi Kato, Takashi Yoshizane, Yoshiaki Goto, Hirotaka Miwa, Hiroto Yagasaki, Ryota Watanabe, Takahiro Ueno, Shuntaro Horio (Gifu Prefectural General Medical Center), Toshiki Tanaka (Gifu University), Yasuhiro Morita (Ogaki Municipal Hospital), and Haruo Kamiya (Japanese Red Cross Nagoya Daiichi Hospital) for their support of this clinical trial.

References

1. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: Experimental observations and clinical implications. *Circulation* 1990; **81:** 1161–1172.
2. Fisher SA, Zhang H, Doree C, Mathur A, Martin-Rendon E. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev* 2015; **9:** CD006536.
3. Kuroda Y, Kitada M, Wakao S, Nishikawa K, Tanimura Y, Makinoshima H, et al. Unique multipotent cells in adult human mesenchymal cell populations. *Proc Natl Acad Sci USA* 2010; **107:** 8639–8643.
4. Wakao S, Kitada M, Kuroda Y, Shigemoto T, Matsuse D, Akashi H, et al. Multilineage-differentiating stress-enduring (muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. *Proc Natl Acad Sci USA* 2011; **108:** 9875–9880.
5. Kuroda Y, Wakao S, Kitada M, Murakami T, Nojima M, Dezawa M. Isolation, culture and evaluation of multilineage-differentiating stress-enduring (muse) cells. *Nat Protoc* 2013; **8:** 1391–1415.
6. Heneidi S, Simerman AA, Keller E, Singh P, Li X, Dumesic DA, et al. Awakened by cellular stress: Isolation and characterization of a novel population of pluripotent stem cells derived from human adipose tissue. *PLoS One* 2013; **8:** e64752.
7. Yamada Y, Wakao S, Kushida Y, Minatoguchi S, Mikami A, Higashi K, et al. S1P-S1PR2 axis mediates homing of Muse cells into damaged heart for long-lasting tissue repair and functional recovery after acute myocardial infarction. *Circ Res* 2018; **122:** 1069–1083.
8. Abe T, Aburakawa D, Niizuma K, Iwabuchi N, Kajitani T, Wakao S, et al. Intravenously transplanted human muse cells afford brain repair in a mouse lacunar stroke model. *Stroke* 2020; **51:** 601–611.
9. Dokainish H, Rajaram M, Prabhakaran D, Afzal R, Orlandini A, Staszewsky L, et al; Echocardiographic Substudy of the OASIS-6 Trial Investigators. Incremental value of left ventricular systolic and diastolic function to determine outcome in patients with acute ST-segment elevation myocardial infarction: The echocardiographic substudy of the OASIS-6 trial. *Echocardiography* 2014; **31:** 569–578.
10. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, Developed in Conjunction with the European Association of Echocardiography, a Branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005; **18:** 1440–1463.
11. Kinoshita K, Kuno S, Ishimine H, Aoi N, Mineda K, Kato H, et al. Therapeutic potential of adipose-derived SSEA-3-positive Muse cells for treating diabetic skin ulcers. *Stem Cells Transl Med* 2015; **4:** 146–155.
12. Wakao S, Akashi H, Kushida Y, Dezawa M. Muse cells, newly found non-tumorigenic pluripotent stem cells, reside in human mesenchymal tissues. *Pathol Int* 2014; **64:** 1–9.
13. Hori E, Hayakawa Y, Hayashi T, Hori S, Okamoto S, Shibata T, et al. Mobilization of pluripotent multilineage-differentiating stress-enduring cells in ischemic stroke. *J Stroke Cerebrovasc Dis* 2016; **25:** 1473–1481.
14. Tanaka T, Nishigaki K, Minatoguchi S, Nawa T, Yamada Y, Kanamori H, et al. Mobilized Muse cells after acute myocardial infarction predict cardiac function and remodeling in the chronic phase. *Circ J* 2018; **82:** 561–571.

Supplementary Files

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-20-0307