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

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**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 × 10⁷ 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). 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. 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.

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. 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 (1.5×10⁷ cells/15 mL of frozen preparation) was manufactured by Life Science Institute, Inc. (Tokyo, Japan). 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. To assess the primary outcome, vital signs were measured, and a medical
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 ± 3.2, 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 allogeneic 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, 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. 

Single-photon emission computed tomography (SPECT) using Tc-MIBI or 99mTc-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.
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.

Table 2. Assessment of Safety of CL2020

| Hematologic tests                  | Baseline      | 2 weeks       | 4 weeks       | 8 weeks       | 12 weeks      |
|------------------------------------|---------------|---------------|---------------|---------------|---------------|
| 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 (10^6/μL)          | 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±836.4 | 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 (10^3/μL)                | 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    |
| 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.
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 3×10⁵ 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.3 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.

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Supplementary Files
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