Safety and tolerability of a multilineage-differentiating stress-enduring cell-based product in neonatal hypoxic-ischaemic encephalopathy with therapeutic hypothermia (SHIELD trial): a clinical trial protocol open-label, non-randomised, dose-escalation trial

Nao Matsuyama 1, Shinobu Shimizu 1, Kazuto Ueda 2, Toshihiko Suzuki 2,3, Sakiko Suzuki 2, Ryosuke Miura 2, Akemi Katayama 1, Masahiko Ando 1, Masaaki Mizuno 1, Akihiro Hirakawa 4, Masahiro Hayakawa 2, Yoshiaki Sato 2

ABSTRACT

Introduction Neonatal hypoxic-ischaemic encephalopathy (HIE) is an important illness associated with death or cerebral palsy. This study aims to assess the safety and tolerability of the allogenic human multilineage-differentiating stress-enduring cell (Muse cell)-based product (CL2020) cells in newborns with HIE. This is the first clinical trial of CL2020 cells in neonates.

Methods and analysis This is a single-centre, open-label, dose-escalation study enrolling up to 12 patients. Neonates with HIE who receive a course of therapeutic hypothermia therapy, which cools to a body temperature of 33°C–34°C for 72 hours, will be included in this study. A single intravenous injection of CL2020 cells will be administered between 5 and 14 days of age. Subjects in the low-dose and high-dose cohorts will receive 1.5 and 15 million cells per dose, respectively. The primary outcome is the occurrence of any adverse events within 12 weeks after administration. The main secondary outcome is the Bayley Scales of Infant and Toddler Development Third Edition score and the developmental quotient per the Kyoto Scale of Psychological Development 2001 at 78 weeks.

Ethics and dissemination This study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The Nagoya University Hospital Institutional Review Board (No. 312005) approved this study on 13 November 2019. The results of this study will be published in peer-reviewed journal and reported in international conferences.

Trial registration numbers NCT04261335, jRCT2043190112.

INTRODUCTION

Neonatal hypoxic-ischaemic encephalopathy (HIE) results from acute perinatal asphyxia and can lead to poor patient outcomes, including death, physical disabilities and mental retardation. HIE has an estimated incidence of 1.5 per 1000 live births (95% CI 1.3 to 1.7) from the three population-based studies in UK, Australia, Sweden carried out since 1980,1 and the incidence of moderate or severe HIE has been reported to be 0.37 per 1000 term live births in Japan.2 Birth asphyxia accounts for 23% of global neonatal deaths.3 Because HIE is associated with irreversible injury to the central nervous system, its sequelae such as cerebral palsy, epilepsy or cognitive impairment could be major persistent burdens on both patients and their families.

The most evidence-based treatment for moderate-to-severe HIE is therapeutic hypothermia, which maintains a body temperature of 33°C–34°C for 72 hours.4,5 However, its effectiveness is limited. A previous study reported that the number needed to treat...
was 9 (95% CI 5 to 25) for hypothermia therapy to avoid one death or severe disability at 18 months. Therefore, a novel treatment for moderate-to-severe HIE is warranted.

Regenerative medicine has been developed as a new and effective treatment for HIE. Preclinical animal studies using umbilical cord blood cells (UCBCs) in neonatal HIE and stroke rat models have reported effectiveness. In addition, some exploratory clinical studies have shown the safety and feasibility of autologous UCBCs administration for HIE neonates. However, preparing autologous UCBCs requires well-equipped facilities and sufficient human resources in birthing centres, clinics or hospitals.

From a wide variety of options as candidates for regenerative cells, we have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent markers that self-renew and differentiate from a single cell into each of the three germ layer cells. They are positive for stage-specific embryonic antigen (SSEA)-3 and CD105 in the peripheral blood, bone marrow and organ connective tissues. Muse cells also have a specific immunomodulatory system, represented by human leucocyte antigen (HLA)-G expression, allowing them to be directly administered without HLA matching or immunosuppressant agents. Furthermore, after intravenous administration, Muse cells are distributed to the damaged site by sphingosine monophosphate (S1P)-S1P receptor 2 axis mechanism, and then self-renewed without artificial differentiation or induction. After migrating, Muse cells differentiate into tissue-compatible cells according to the microenvironment and remain integrated into the host tissue to participate in tissue repair. Based on these characteristics, intravenous administration of allogenic Muse cells is expected to be an effective regenerative therapy for HIE.

We found that the systemic administration of human Muse cells in the perinatal HIE rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left carotid artery, improved learning deficits and motor impairment. In addition, human Muse cells are localised in the damaged brain and differentiate into neurons. These effects were much clearer in the Muse cells than in mesenchymal stem cells (MSCs) without Muse cells subpopulation. Moreover, we confirmed that the human allogenic Muse cells-based product, CL2020, manufactured by Life Science Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical Holdings, exerted a therapeutic effect with no toxicity initiated clinical trial on neonatal HIE to investigate the safety, tolerability and efficacy in neurodevelopmental outcomes at 18 months. This clinical trial is named ‘The Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring cell-based product cells in Neonatal Hypoxic-Ischemic Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation Clinical Trial’ (the SHIELD trial).

METHODS AND ANALYSIS

Objective and study design

The SHIELD trial’s main objective is to confirm the safety and tolerability of intravenous CL2020 cells in neonates with HIE. This trial is a single-centre, open-label, non-randomised, dose-escalation exploratory clinical trial. We have planned a standard 3+3 dose-escalation design to examine the optimal dose of CL2020 cells for neonatal safety and tolerability. The follow-up period is up to 78 weeks after administering CL2020 cells to each patient.

Recruitment and setting

Patient recruitment is done in Nagoya University Hospital or by receiving referrals of patients from other hospitals in our district. The investigators will obtain written informed consent from the patients’ legal parental authority before screening. After screening and verifying the patients’ eligibility, they will be registered for the trial.

Participants

We will recruit a maximum of 12 neonates with HIE who have received therapeutic hypothermia. They must meet the following inclusion criteria:

- At least 36 weeks gestational age, and one of the following criteria:
  - Apgar score ≤5 at 10 min.
  - Continued neonatal resuscitation for at least 10 min.
  - pH <7.0, or base deficit ≥16 mmol/L in any blood sample obtained within 60 min after birth.
- Moderate or severe encephalopathy, as judged using the Sarnat criteria.

SARS-CoV-2 infection (jRCT2043210005). The first-in-human clinical trial for acute myocardial infarction was performed in 3 patients and indicated that CL2020 was safe and significantly improved the left ventricular ejection fraction. A phase 1/2 open-label trial on adult epidermolysis bullosa was also recently published. A total of five patients received a single injection of CL2020, and the ulcer size was significantly reduced for up to 3 months.

Nevertheless, the safety and tolerability of Muse cells in neonates are unknown because they have never been administered to neonates. Based on these results, we planned the first-in-neonate clinical trial to confirm the safety and tolerability of CL2020 cells in patients with moderate-to-severe HIE receiving hypothermia therapy. Hence, we describe the detailed design of an investigator-initiated clinical trial on neonatal HIE to investigate the safety, tolerability and efficacy in neurodevelopmental outcomes at 18 months. This clinical trial is named ‘The Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring cell-based product cells in Neonatal Hypoxic-Ischemic Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation Clinical Trial’ (the SHIELD trial).
Therapeutic hypothermia initiated within 6 hours after birth and continued for 72 hours.
- Birth weight ≥1800 g.
- Heart rate ≥100 /min and SpO₂ ≥90% on enrolment.
- Able to provide voluntary informed consent after receiving information about the study (consent will be obtained from a legal proxy).

Exclusion criteria are:
- Suspected or confirmed severe congenital abnormalities or chromosomal anomaly.
- Planned to undergo surgery or radiation therapy.
- Scheduled to take systemic corticosteroids treatment for over 5 days.
- Blood glucose ≥200 mg/dL continuously sustained.
- Participation in another interventional clinical study.
- Suspected or confirmed active and severe infection.
- Positive for hepatitis B virus surface (HBs) antigen, hepatitis type C virus (HCV) antibody, human immunodeficiency virus (HIV) antibody, human T-cell leukemia virus (HTLV)-1 antibody or syphilis serum reaction.
- History of severe hypersensitivity or anaphylactic reaction.
- Severe complications not related to HIE.

**Patient and public involvement**

Patients’ guardians or members of the public were not involved in this study protocol planning.

**Intervention and follow-up**

The clinical-grade Muse cell-based product, CL2020 (1.5×10⁸ cells/15 mL of frozen preparation), was produced from human allogenic MSCs by LSII. 26 The mediated by RNA transcription,27 we thought that they

Study framework

This is a schematic diagram of this clinical trial as a 3+3 design. It shows the schedule of enrolment, timing of CL2020 cells administration and assessments and visits for each patient, and timing of the DSMB meeting. The DSMB meets for the safety evaluation 4 weeks after CL2020 cells administration to the first patient in each cohort and 12 weeks after administration to the third patient in each cohort to confirm if the remaining participants can be enrolled.

**Study endpoints**

The primary outcome is the incidence of adverse events until 12 weeks after administration. The secondary outcomes are as follows:
- Incidence of composite endpoints (death, continuous respiratory support, or continuous use of vasopressors or pulmonary vasodilators).
- Mortality and overall survival.
- Duration of continuous respiratory support: The definition of respiratory support is the status of conducting artificial ventilation with tracheal intubation.
- Duration of continuous use of vasopressors or pulmonary vasodilators: dopamine, dobutamine, epinephrine, norepinephrine, milrinone, vasopressin, diltiazem hydrochloride, lisinopril hydrochloride, nitric oxide, epoprostenol sodium, nitroglycerin and alprostadil alfadex.
- The Bayley Scales of Infant and Toddler Development Third Edition score at 78 weeks.
- Developmental quotient as per the at 78 weeks.
- Assessment of postnatal development such as head control, rolling, sitting, crawling, unaided walking and saying several meaningful words.
- Presence of spasticity: The definition of spasticity is the status of increased muscle tone or increased deep tendon reflex.
- Presence of epilepsy: The definition of epilepsy is based on the International League Against Epilepsy. 30
- MRI score: The scoring system is based on the report of Barkovich et al. 31
- The score of Expanded and Revised Gross Motor Function Classification System at 78 weeks.

In addition, we will collect vital signs and laboratory values for safety assessment at specific points, as shown...
in table 1. In addition, tolerability is determined by the investigator based on the suggestion of the DSMB by confirming a serious adverse event related to the administration of the investigational product.

**Sample size calculation**

We did not calculate the sample size with statistical rationale because we used a 3+3 dose-escalation design to confirm the safety and tolerability of CL2020 cells. The scheduled number of enrolled patients is 12.

**Statistical analysis**

All analyses are based on an intention-to-treat principle. We will summarise the demographic data using descriptive statistics. The main purpose of this exploratory clinical trial is ‘to confirm the safety and tolerability’ of the Muse cell product. Therefore, we will analyse adverse events on the safety analysis set defined as all subjects enrolled in this study and received the investigational cell product. All adverse events will be confirmed for the primary endpoint, and the proportions of the adverse events and their 95% CI based on the Clopper-Pearson method will be calculated. Overall survival, defined as the time from birth to the date of death due to any cause, will be summarised using the Kaplan-Meier method. Descriptive statistics for continuous variables and frequency and proportion for categorical variables will be calculated for each secondary endpoint. Depending on the endpoint (eg, the duration of continuous respiratory support, continuous use of vasopressors or pulmonary vasodilators), it will be summarised excluding patients who had been using these therapies prior to the cells administration as necessary. Statistical analysis will be performed using the SAS software (SAS Institute, V.9.4). Statistical significance will be defined as p<0.05. Although some endpoints, including the provision of respiratory support and the use of vasoactive drugs, may be affected by pre-enrolment condition, the effects of these potential baseline differences will not be adjusted in the analysis.

**Monitoring and auditing**

The monitoring personnel will investigate the progress of this trial and confirm the adequacy of the research procedures. The auditing personnel will check the quality of this trial independent of the investigators, according to the laws, regulations, study protocol and standard operating procedures.

**Status of this trial**

The Ministry of Health, Labour and Welfare accepted this clinical trial notification as a trial on a new cellular and tissue-based product in January 2020. The first participant was registered, and CL2020 cells were administered in March 2020. Three patients were enrolled into a low-dose cohort, while six were allocated to a high-dose cohort as of July 2021. Patient recruitment was performed in Nagoya.
**Table 1** Schedule of interventions and assessments

<table>
<thead>
<tr>
<th>Treatments and assessments</th>
<th>Registration</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 12</th>
<th>Week 26</th>
<th>Week 38</th>
<th>Week 52</th>
<th>Week 78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreement</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographics, current medications</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Registration</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assignment</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Administration</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs*</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Oxygen saturation</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Haematological tests†</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical tests‡</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine analysis§</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite endpoints</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spasticity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postnatal development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bayley scale¶</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kyoto scale**</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMFCS</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Blood pressure, pulse rate and body temperature.
†Red cell count, haemoglobin, haematocrit, white cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monocytes) and platelet count.
‡Blood urea nitrogen, creatinine, lactate dehydrogenase, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, gamma-glutamyl transpeptidase, total bilirubin, direct bilirubin, creatine kinase, C reactive protein, sodium, potassium, calcium, phosphorus and blood glucose level.
§pH, urine protein, urine occult blood and urine sugar.
¶Bayley Scales of Infant and Toddler Development Third edition.
**Kyoto Scale of Infant and Toddler Development Third edition.
††GMFCS, Gross Motor Function Classification System.
University Hospital from February 2020 to July 2021, and the study will be terminated in September 2023.

ETHICS AND DISSEMINATION

Ethical approval
This study was approved by the Nagoya University Hospital Institutional Review Board (No. 312005) on 13 November 2019. This study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The investigators must always obtain approval from the Institutional Review Board about any amendment to the protocol and provide the necessary reasons.

Patient consent for participation
The investigators and trained clinical research coordinators will introduce the trial to patients’ legal representatives with prepared information sheets and informed consent forms (online supplemental file 1). The investigator will obtain written consent to participate in the trial. Subjects will be identified during the data collection using a subject identification code. All personnel involved in this study will take the best possible precautions to ensure the protection of patients’ personal information.

Dissemination
The results of this clinical trial will be published in peer-reviewed journals, presented in conferences and submitted to clinical trial registries.

DISCUSSION
This clinical trial aims to evaluate the safety and tolerability of CL2020 (a Muse cell-based product) cells in neonates. When CL2020 cells were administered intravenously to infant rats, the cells were distributed mainly in the lungs immediately after administration. However, there was no change in respiratory condition or pathological evaluation. Based on non-clinical study data and ongoing clinical trials of CL2020, we decided to implement this clinical trial to ensure safety in neonates.

Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy. Several randomised controlled trials of hypothermia therapy for HIE have been conducted, and hypothermia is currently the sole neuroprotective therapy. However, its effectiveness is insufficient, and a novel therapy is required. Regenerative therapy is the focus of next-generation therapy. Clinical studies with autologous UCBCs for HIE had been conducted before the development of CL2020. Here, we present the overall design of this single-centre, open-label, dose-escalation clinical trial of Muse cell products in HIE patients with hypothermia. This clinical trial is the first clinical application of CL2020 cells in neonates based on our non-clinical study results. If we can verify that this product is safe and well tolerable in neonates, its application may expand to other disorders in neonates and children.

Acknowledgements
The authors are grateful to LSII for providing the CL2020. We would like to thank all the physicians who referred patients for this study and the staff at Nagoya University Hospital for assisting with the recruitment and evaluation of patients for this trial. We thank the DSMB members for evaluating the safety data in this study. We would like to thank Editage (www.editage.com) for English language editing.

Contributors
YS is the principal investigator in this trial and has access to all data. NM, SSh, KU, TS, AK, MA, AH and YS developed the study protocol. SSh and YS participated in the conception and design of the study. AK is a quality control monitor, MA is responsible for data management, and AH supervises the statistical analysis. NM, SSh and MM supported the preparation and management of this study. KU, TS, SSh, RM, MH and YS helped recruit and evaluate patients and prepare cells for administration. NM drafted and revised the manuscript. SSh and YS have revised the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the Japan Agency for Medical Research Development (grant number: JP21lm0203143).

Competing interests
SSh, MM, and YS have collaborative projects with research funding from LSII for perinatal diseases. SSh and AH receive fees based on a consultation contract from LSII. SSh, TS, MM, MH, and YS have a patent for the application of Muse cells in the treatment of HIE and other indications. LSII provided CL2020 for this clinical trial free of charge.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication
Not applicable.

Provenance and peer review
Not commissioned; externally peer reviewed.

Supplemental material
This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s).
of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Nao Matsuyama http://orcid.org/0000-0002-0096-0832
Shinobu Shimizu http://orcid.org/0000-0001-9666-3489
Toshikiko Suzuki http://orcid.org/0000-0001-8760-1404
Sakiko Suzuki http://orcid.org/0000-0003-2114-9499
Yoshiaki Sato http://orcid.org/0000-0001-6320-9176

REFERENCES


