






# BMJ Open 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 <sup>1</sup>, Shinobu Shimizu <sup>1</sup>, Kazuto Ueda,<sup>2</sup> Toshihiko Suzuki <sup>2,3</sup>, Sakiko Suzuki <sup>2</sup>, Ryosuke Miura,<sup>2</sup> Akemi Katayama,<sup>1</sup> Masahiko Ando,<sup>1</sup> Masaaki Mizuno,<sup>1</sup> Akihiro Hirakawa,<sup>4</sup> Masahiro Hayakawa,<sup>2</sup> Yoshiaki Sato <sup>2</sup>

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For numbered affiliations see end of article.

## Correspondence to

Dr Yoshiaki Sato;  
yoshiaki@med.nagoya-u.ac.jp and  
Mr Shinobu Shimizu;  
s-shimizu@med.nagoya-u.ac.jp

## 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,

## Strengths and limitations of this study

- This is the first clinical trial in neonates aimed at the safety and tolerability of CL2020 cells, a Muse cell-based product.
- Further investigation will be needed to confirm the efficacy and safety of this products in neonatal hypoxic-ischaemic encephalopathy (HIE) after this clinical trial.
- If this product is found to be safe and well tolerated by neonates with HIE, its application may expand to other disorders in neonates and children.

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,<sup>1</sup> and the incidence of moderate or severe HIE has been reported to be 0.37 per 1000 term live births in Japan.<sup>2</sup> Birth asphyxia accounts for 23% of global neonatal deaths.<sup>3</sup> 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.<sup>4,5</sup> 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.<sup>6</sup> 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.<sup>7-9</sup> In addition, some exploratory clinical studies have shown the safety and feasibility of autologous UCBCs administration for HIE neonates.<sup>10,11</sup> 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,<sup>12-15</sup> we have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells are endogenous, non-tumourigenic, 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.<sup>16</sup> They are positive for stage-specific embryonic antigen (SSEA)-3 and CD105 in the peripheral blood, bone marrow and organ connective tissues.<sup>17,18</sup> 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.<sup>19</sup> Furthermore, after intravenous administration, Muse cells are distributed to the damaged site by sphingosine monophosphate (S1P)-S1P receptor 2 axis mechanism,<sup>19</sup> 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.<sup>20,21</sup> 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.<sup>22</sup> 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 in the HIE rat models. To verify the safety and effectiveness of CL2020, LSII has conducted several clinical trials in adult patients with acute myocardial infarction (JapicCTI-No.: JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103), epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841), amyotrophic lateral sclerosis (jRCT2063200047) and acute respiratory distress syndrome associated with

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.<sup>23</sup> 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.<sup>24</sup>

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).

## 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  $\leq 5$  at 10 min.
  - Continued neonatal resuscitation for at least 10 min.
  - pH  $< 7.0$ , or base deficit  $\geq 16$  mmol/L in any blood sample obtained within 60 min after birth.
- ▶ Moderate or severe encephalopathy, as judged using the Sarnat criteria.<sup>25</sup>

- ▶ Therapeutic hypothermia initiated within 6 hours after birth and continued for 72 hours.
- ▶ Birth weight  $\geq 1800$  g.
- ▶ Heart rate  $\geq 100$  /min and  $\text{SpO}_2 \geq 90\%$  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  $\geq 200$  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 ( $1.5 \times 10^7$  cells/15 mL of frozen preparation), was produced from human allogenic MSCs by LSII.<sup>26</sup> The CL2020 was produced by exposing MSCs to some stressors, and they were enriched to be positive for both SSEA-3 and CD105 but negative for CD45. We will prepare cells from CL2020 for administration to neonates by centrifuging the product after thawing, removing the supernatant and suspending with acetated Ringer's solution. The patients will receive the prepared cells intravenously once between 5 and 14 days after birth. We decided to administer as soon as possible within this window (5–14 days after birth) in principle after registration. This study will use a 3+3 dose-escalation design, setting two cohorts for the injected dose. Subjects in the low-dose cohort will receive 1.5 million cells, while those in the high-dose cohort will receive 15 million cells. The following treatments will be prohibited during the study: corticosteroids (prednisolone converted at 2 mg/kg/day or more, and more than 5 days), other human MSC products, processed cell products except for the red blood cells, other investigational products and the use of investigational medical devices. Regarding corticosteroid, it affects cell proliferation mediated by RNA transcription,<sup>27</sup> we thought that they could affect the function of the administered cells. The data and safety monitoring board (DSMB) will consist of three specialists in paediatric and perinatal care independent of the trial investigators. The DSMB will be held at

predefined times in both cohorts: at 4 weeks after administering to the first patient and 12 weeks after administering to the third patient in each cohort. The council will also be held when a product-related severe adverse event occurs or when investigators consider that it should be convened due to safety concerns. The DSMB will recommend whether this trial should be moved forward or be discontinued. [Figure 1](#) illustrates the framework of this study. The study participants will be hospitalised for at least 2 weeks after CL2020 cells administration and followed up for 78 weeks. The planned visits and data collection are presented in [table 1](#).

### Study framework

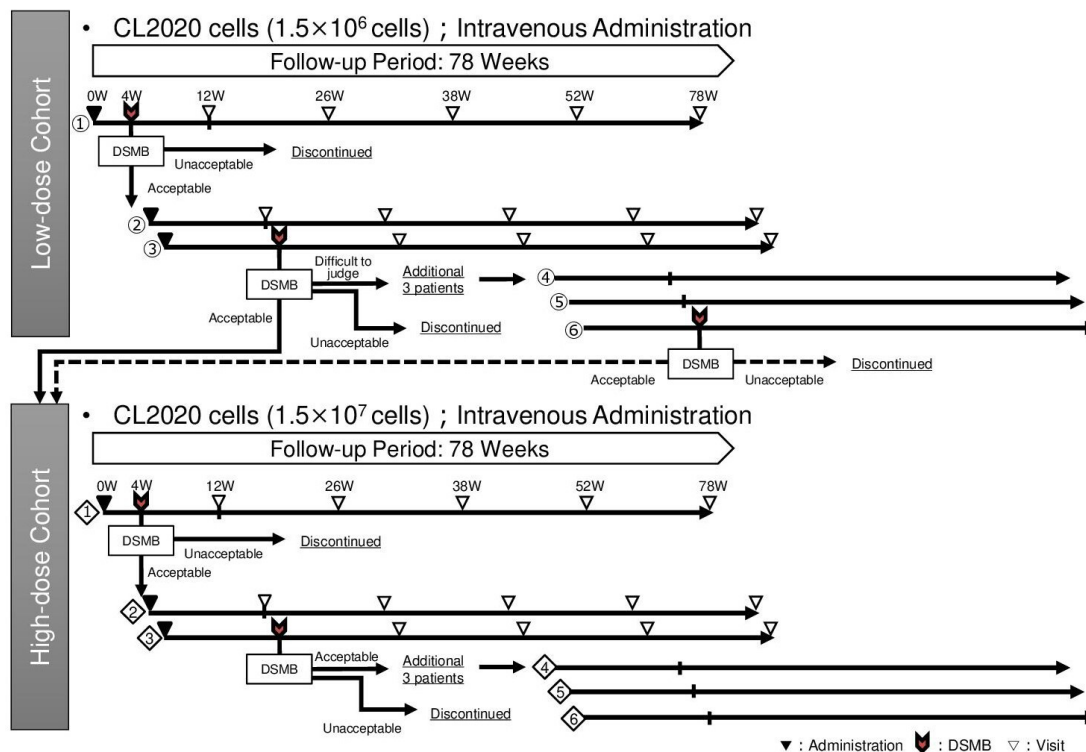
This is a schematic diagram of this clinical trial as a 3+3 design. It shows the schedule of enrolment, timing of CL2020 cells administration and assessments and visits for each patient, and timing of the DSMB meeting. The DSMB meets for the safety evaluation 4 weeks after CL2020 cells administration 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, *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide, epoprostenol sodium, nitroglycerin and alprostadil alfadex.
- ▶ The Bayley Scales of Infant and Toddler Development Third Edition<sup>28</sup> score at 78 weeks.
- ▶ Developmental quotient as per the<sup>29</sup> 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.<sup>30</sup>
- ▶ MRI score: The scoring system is based on the report of Barkovich *et al.*<sup>31</sup>
- ▶ The score of Expanded and Revised Gross Motor Function Classification System<sup>32</sup> at 78 weeks.

In addition, we will collect vital signs and laboratory values for safety assessment at specific points, as shown



**Figure 1** 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, assessments and visits for each patient, and timing of the data safety monitoring board (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. DSMB, data and safety monitoring board.

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

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

\*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 Psychological Development 2001.

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 was 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,<sup>33–38</sup> 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.<sup>10 11</sup> This UCBCs therapy requires additional equipment and human resources for its preparation because the newborns' umbilical cord blood has to be collected at birth, and the patients receive the first dose of prepared UCBCs within 24 hours after birth. In contrast, in our non-clinical study, single intravenous administration of Muse cells to HIE model rats 3 days after hypoxic-ischaemic injury ameliorated behavioural abnormalities up to 5 months.<sup>22</sup> In a non-clinical study using CL2020 cells, the treatment effect was

exerted at even 7 days after insult by hypoxic ischaemia. In addition, a single dose of CL2020 cells administered via the vein at the subacute (about 9 days after onset) and chronic phases (about 30 days) was effective in a mouse lacunar stroke model.<sup>26</sup> Thus, we set the administration of Muse cells to human neonates between 5 and 14 days after birth, which means that physicians and patients' families can afford the time to decide or prepare the treatment based on the patient's condition or seek other opinions.

We held a consultation meeting about the main clinical trial design, including the timing of administration as above with the Japanese regulatory authority, Pharmaceutical and Medical Devices Agency, and they agreed to our proposed design for this trial. We will perform a randomised placebo-controlled clinical trial to evaluate the effectiveness of CL2020 cells for HIE after confirming the safety and tolerability of its intravenous administration in neonates.

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.

### Author affiliations

<sup>1</sup>Department of Advanced Medicine, Nagoya University Hospital, Nagoya, Japan

<sup>2</sup>Division of Neonatology, Center for Maternal-Neonatal Care, Nagoya University Hospital, Nagoya, Japan

<sup>3</sup>Division of Neonatology, Tokyo Women's Medical University Medical Center East, Arakawa-ku, Tokyo, Japan

<sup>4</sup>Department of Clinical Biostatistics, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

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**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, SSu, 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.

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**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.

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**Patient consent for publication** Not applicable.

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#### ORCID iDs

Nao Matsuyama <http://orcid.org/0000-0002-0096-0832>

Shinobu Shimizu <http://orcid.org/0000-0001-9066-3489>

Toshihiko 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>

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