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# BMJ Open

## Safety and tolerability of CL2020 in neonatal hypoxic-ischemic encephalopathy patients with therapeutic hypothermia (SHIELD trial): a clinical trial protocol for open-label, non-randomized, dose-escalation study

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Manuscripts

1 **Safety and tolerability of CL2020 in neonatal hypoxic-ischemic**  
2 **encephalopathy patients with therapeutic hypothermia (SHIELD trial): a**  
3 **clinical trial protocol for open-label, non-randomized, dose-escalation study**

4  
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26  
27 25 **Keywords**

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30 26 Hypoxic-ischemic encephalopathy, neonates, cerebral palsy, hypothermia,

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33 27 mesenchymal stem cell, Muse cell

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39 29 **Protocol version 2.0** (April 21, 2021)

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42 30 **Word count** 2,755 words

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48 32 **Abstract**

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51 33 **Introduction** Neonatal hypoxic-ischemic encephalopathy (HIE) is an important

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54 34 illness associated with death or cerebral palsy. This study aims to assess the

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6 35 safety and tolerability of the allogenic human multilineage-differentiating stress-  
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9 36 enduring cell (Muse cell)-based product, CL2020, in newborns with HIE. This is the  
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12 37 first clinical trial of CL2020 for neonates.  
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15 38 **Methods and analysis** This is a single-center, open-label, dose-escalation study  
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18 39 enrolling up to 12 patients. Neonates with HIE who receive proper hypothermia  
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21 40 therapy will be included in this study. A single intravenous injection of CL2020 will  
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24 41 be administered between 5 and 14 days of age. Subjects in the low-dose and high-  
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27 42 dose cohorts will receive 1.5 million cells and 15 million cells per dose,  
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29  
30 43 respectively. The primary outcome is the incidence of any adverse events until 12  
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33 44 weeks after administration. The main secondary outcome is the Bayley Scales of  
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36 45 Infant and Toddler Development Third Edition score and the developmental  
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39 46 quotient as per the Kyoto Scale of Psychological Development 2001 at 78 weeks.  
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42 47 **Ethics and dissemination** This study will be conducted in accordance with the  
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45 48 Declaration of Helsinki and Good Clinical Practice. The Nagoya University Hospital  
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48 49 Institutional Review Board (No. 312005) approved this study on November 13,  
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51 50 2019.  
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54 51 **Trial registration** ClinicalTrials.gov: NCT04261335, registered on 7 February  
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7 52 2020, <https://clinicaltrials.gov/ct2/show/NCT04261335>. Japan Registry of Clinical  
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9 53 Trials: jRCT2043190112, registered on 6 February 2020,  
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12 54 <https://jrct.niph.go.jp/latest-detail/jRCT2043190112>  
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### 18 56 **Strengths and limitations of this study**

- 21 57 • This is the first clinical trial aiming at the safety and tolerability of CL2020, a Muse  
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24 58 cell-based product, in neonates.
- 27 59 • Hypothermia is currently the sole neuroprotective therapy for HIE; however, its  
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30 60 effectiveness is insufficient, and a novel therapy is therefore required.
- 33 61 • After confirming the safety and tolerability of intravenous CL2020 administration  
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36 62 in neonates, we will need a randomized placebo-controlled clinical trial to evaluate  
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39 63 the effectiveness of CL2020 for HIE.
- 42 64 • This clinical trial is the first clinical application in neonates based on our non-  
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45 65 clinical study results; if this product is safe and well-tolerated by neonates in this  
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48 66 study, this may expand its application to other disorders in neonates and children.

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54 68

## 69 Introduction

70 Neonatal hypoxic-ischemic encephalopathy (HIE) results from acute perinatal  
71 asphyxia and can lead to poor patient outcomes, including death, physical  
72 disabilities, and mental retardation. HIE is expected to occur in 1.5 per 1,000 live  
73 births (95% confidence intervals [CI]: 1.3 to 1.7) globally,[1] and the incidence of  
74 moderate or severe HIE has been reported to be 0.37 per 1,000 term live births in  
75 Japan.[2] Birth asphyxia accounts for 23% of global neonatal deaths.[3] Because  
76 HIE is associated with irreversible injury to the central nervous system, its  
77 sequelae such as cerebral palsy, epilepsy, or cognitive impairment could be a  
78 persistent major burden on both patients and their families.

79 The most evidence-based treatment for moderate-to-severe HIE is therapeutic  
80 hypothermia, which maintains a body temperature of 33–34°C for 72 hours[4, 5];  
81 however, its effectiveness is limited. A previous study reported that the number  
82 needed to treat was 9 (95% CI: 5–25) for hypothermia therapy to avoid 1 death or  
83 severe disability at 18 months.[6] Therefore, a novel treatment for moderate-to-  
84 severe HIE is warranted.

85 Regenerative medicine has been developed as a new and effective treatment for

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7 86 HIE. Preclinical animal studies using umbilical cord blood cells (UCBCs) in  
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10 87 neonatal HIE and stroke rat models have reported effectiveness.[7–9] In addition,  
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12 88 some exploratory clinical studies have shown the safety and feasibility of  
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15 89 autologous UCBCs administration for HIE neonates.[10, 11] However, the  
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18 90 preparation of autologous UCBCs requires well-equipped facilities and sufficient  
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21 91 human resources in birthing centers, clinics, or hospitals.  
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24 92 From a wide variety of options as candidates for regenerative cells,[12–15] we  
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27 93 have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse  
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30 94 cells are endogenous, non-tumorigenic, pluripotent-like stem cells positive for  
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33 95 pluripotent markers, that self-renew and differentiate from a single cell into each of  
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36 96 the three germ layer cells.[16] They are positive for both stage-specific embryonic  
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39 97 antigen (SSEA)-3 and CD105 in the peripheral blood, bone marrow, and organ  
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42 98 connective tissues.[17, 18] Muse cells also have a specific immunomodulatory  
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45 99 system, represented by human leukocyte antigen (HLA) -G expression, allowing  
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48 100 them to be directly administered without HLA matching or immunosuppressant  
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51 101 agents.[19] Furthermore, after intravenous administration, Muse cells are  
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54 102 distributed to the damaged site by sphingosine monophosphate (S1P)-sphingosine  
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6 103 monophosphate receptor 2 (S1PR2) axis mechanism,[19] and then self-renewed  
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9 104 without artificial differentiation or induction. After migrating, Muse cells differentiate  
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12 105 into tissue-compatible cells according to the microenvironment and remain  
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15 106 integrated in the host tissue to participate in tissue repair.[20, 21] Based on these  
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18 107 characteristics, intravenous administration of allogenic Muse cells are expected to  
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21 108 be an effective regenerative therapy for HIE.  
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24 109 We found that the systemic administration of human Muse cells for the perinatal  
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27 110 HIE rat model, made by 60 min of hypoxic (8%) exposure following ligation of the  
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30 111 left carotid artery, improved learning deficits and motor impairment. In addition,  
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33 112 human Muse cells are localized in the damaged brain and differentiate into  
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36 113 neurons. These effects were much clearer in the Muse cells than in MSCs without  
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39 114 Muse cells subpopulation.[22] Moreover, we confirmed that the human allogenic  
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42 115 Muse cells-based product, CL2020, manufactured by Life Science Institute, Inc.  
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45 116 (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical Holdings  
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48 117 Corporation, exerted a treatment effect with no toxicity in the HIE rat models. To  
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51 118 verify the safety and effectiveness of CL2020, LSII has conducted several clinical  
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54 119 trials with adult patients, namely, acute myocardial infarction (JapicCTI-No.:

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6 120 JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103), epidermolysis  
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9 121 bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841), amyotrophic  
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12 122 lateral sclerosis (jRCT2063200047), and acute respiratory distress syndrome  
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15 123 associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-human  
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18 124 clinical trial for acute myocardial infarction was performed in 3 patients and  
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21 125 indicated that CL2020 was safe and significantly improved the left ventricular  
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24 126 ejection fraction.[23] A phase 1/2 open-label study for adult epidermolysis bullosa  
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27 127 was also recently published. Five patients received a single injection of CL2020,  
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30 128 and the ulcer size was significantly reduced for up to 3 months.[24]  
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33 129 Nevertheless, the safety and tolerance of Muse cells in neonates are unknown  
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36 130 because they have never been administered to neonates. Based on these results,  
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39 131 we planned the first-in-neonate clinical trial to confirm the safety and tolerability of  
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42 132 CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy.  
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45 133 Hence, we describe the detailed design of an investigator-initiated clinical trial on  
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48 134 neonatal HIE to investigate the safety, tolerability, and efficacy in  
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51 135 neurodevelopmental outcomes at 18 months. This clinical trial was named "The  
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54 136 Evaluation of Safety and Tolerability of CL2020 in Neonatal Hypoxic Ischemic  
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6 137 Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation

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9 138 Clinical Trial" (the SHIELD trial).

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14 **Methods and analysis**

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17 **Objective and study design**

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20 142 The SHIELD trial's main objective is to confirm the safety and tolerability of

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23 143 intravenous CL2020 in neonates with HIE. This trial is a single-center, open-label,

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26 144 non-randomized, dose-escalation exploratory clinical trial. We have planned a

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29 145 standard 3 + 3 dose-escalation design to examine the optimal dose of CL2020 for

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32 146 neonatal safety and tolerability. The follow-up period is up to 78 weeks after the

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35 147 administration of CL2020 for each patient.

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41 **Recruitment and setting**

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44 150 Patient recruitment is performed in our hospital or by receiving referrals of patients

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47 151 from other hospitals in our district. Prior to the screening assessment, the

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50 152 investigators will obtain written informed consent from the patients' legal parental

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53 153 authority. After conducting the screening assessment and verifying the patients'

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6 154 eligibility, they will be registered for the trial.  
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## 12 156 **Participants**

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15 157 We will recruit a maximum of 12 neonates with HIE who have received therapeutic  
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18 158 hypothermia. They must meet the following inclusion criteria:

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21 159 1) At least 36 weeks gestational age, and one of the following criteria (i –iii.)

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24 160 i. Apgar score  $\leq 5$  at 10 minutes

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27 161 ii. Continued neonatal resuscitation for at least 10 minutes

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30 162 iii. pH  $< 7.0$ , or base deficit  $\geq 16$  mmol/L in any blood sample obtained  
31  
32  
33 163 within 60 min after birth

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36 164 2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]

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39 165 3) Undergone therapeutic hypothermia started within 6 hours after birth and

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42 166 continued for 72 hours

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45 167 4) Birth weight  $\geq 1,800$  g

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48 168 5) Heart rate  $\geq 100$ / min, and SpO<sub>2</sub>  $\geq 90\%$

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51 169 6) Able to provide voluntary informed consent after receiving information about  
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54 170 the study (consent will be obtained from a legal proxy).  
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9 172 Participants will be excluded according to the following exclusion criteria:

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12 173 1) Suspected or confirmed severe congenital abnormalities or chromosomal

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15 174 anomaly

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18 175 2) Planned to undergo surgery or radiation therapy

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21 176 3) Scheduled to take systemic corticosteroids treatment for over 5 days

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24 177 4) Blood glucose  $\geq$  200 mg/dL continuously sustained

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26  
27 178 5) Participation in another interventional clinical study

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30 179 6) Suspected or confirmed active and severe infection

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33 180 7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or

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36 181 syphilis serum reaction

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39 182 8) History of severe hypersensitivity or anaphylactic reaction

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42 183 9) Severe complications

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48 185 **Patient and public involvement**

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51 186 Patients' guardians, or members of the public were not involved, in this study

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54 187 protocol planning.

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188189 **Intervention and follow-up**

190 The clinical-grade Muse cell-based product, CL2020, ( $1.5 \times 10^7$  cells/ 15 mL of  
191 frozen preparation) was produced from human allogenic MSC by LSII.[26] We will  
192 prepare cells from CL2020 for administration in neonates by centrifuging the  
193 product after thawing, removing the supernatant, and suspending with acetated  
194 Ringer's solution as 15 million cells in 15 mL. The patients will receive the prepared  
195 cells intravenously once between 5 and 14 days after birth. This study will utilize a  
196 3 + 3 dose-escalation design, setting 2 cohorts for the injected dose. Subjects in  
197 the low-dose cohort will receive 1.5 million cells in 1.5 mL for 2 min, whereas  
198 subjects in the high-dose cohort will receive 15 million cells in 15 mL for 20  
199 minutes. The following treatments will be prohibited during the study:  
200 corticosteroids (prednisolone converted at 2 mg/kg/day or more, and more than 5  
201 days), other human mesenchymal stem cell products, processed cell products,  
202 other investigational products, and use of investigational medical devices. The data  
203 and safety monitoring board (DSMB) will consist of 3 specialists in pediatric and  
204 perinatal care independent of the trial investigators. It will be held at predefined

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6 205 times in both cohorts: at 4 weeks after administering to the first patient and 12  
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9 206 weeks after administering to the third patient in each cohort. The council will also  
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12 207 be held when a product-related severe adverse event occurs, or investigators  
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15 208 consider that it should be convened due to safety concerns. The DSMB will  
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18 209 recommend whether this trial should be moved forward or be discontinued. Figure  
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21 210 1 illustrates the framework of this study. The study patients will be hospitalized for  
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24 211 at least 2 weeks after administration and followed-up for 78 weeks. The planned  
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27 212 visits and data collection are presented in Table 1.  
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213 **Table 1**  
 214 **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														
Hospitalization																
Vital signs <sup>a</sup>	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				
Hematological tests <sup>b</sup>	x	x	x	x	x	x	x	x	x		x	x				
Biochemical tests <sup>c</sup>	x	x	x	x	x	x	x	x	x		x	x				
Urine analysis <sup>d</sup>	x	x	x		x	x										
Composite endpoints																
Spasticity								x	x	x	x	x				
Postnatal development									x	x	x	x				
Epilepsy																
MRI	x					x						x				



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Bayley scale <sup>e</sup>	x
Kyoto scale <sup>f</sup>	x
GMFCS <sup>g</sup>	x

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<sup>a</sup>Blood pressure, pulse rate, and body temperature

<sup>b</sup>Red blood cell count, hemoglobin, hematocrit, white blood cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monocytes), and platelet count

<sup>c</sup>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.

<sup>d</sup>pH, urine protein, urine occult blood, and urine sugar

<sup>e</sup>Bayley Scales of Infant and Toddler Development Third edition

<sup>f</sup>Kyoto Scale of Psychological Development 2001

<sup>g</sup>Expanded and Revised Gross Motor Function Classification System

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216 **Study endpoints**

217 The primary outcome is the incidence of adverse events until 12 weeks after  
218 administration. The secondary outcomes are set as follows:

- 219 1) Incidence of composite endpoints (death, continuous respiratory support, or  
220 continuous use of vasopressors or pulmonary vasodilators)
- 221 2) Mortality and overall survival
- 222 3) Duration of continuous respiratory support: The definition of respiratory  
223 support is the status of conducting artificial ventilation with tracheal  
224 intubation
- 225 4) Duration of continuous use of vasopressors or pulmonary vasodilators;  
226 dopamine, dobutamine, adrenaline, noradrenaline, milrinone, vasopressin,  
227 *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide,  
228 epoprostenol sodium, nitroglycerin, and alprostadil alfadex
- 229 5) The Bayley Scales of Infant and Toddler Development Third Edition [27]  
230 score at 78 weeks
- 231 6) The developmental quotient as per Kyoto Scale of Psychological  
232 Development 2001[28] at 78 weeks

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6 233 7) Assessment of postnatal development such as head control, rolling, sitting,  
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9 234 crawling, walking unaided, and saying several meaningful words  
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12 235 8) Presence of spasticity: The definition of spasticity is the status of increased  
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14  
15 236 muscle tone or increased deep tendon reflex  
16  
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18 237 9) Presence of epilepsy: The definition of epilepsy is based on the  
19  
20  
21 238 International League Against Epilepsy[29]  
22  
23  
24 239 10) Magnetic resonance imaging score: The scoring system is based on the  
25  
26  
27 240 report of Barkovich et al.[30]  
28  
29  
30 241 11) The score of Expanded and Revised Gross Motor Function Classification  
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33 242 System[31] at 78 weeks  
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36 243 In addition, we will collect vital signs and laboratory values for safety  
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39 244 assessment at specific points, as shown in Table 1.  
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#### 45 246 **Sample size calculation**

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48 247 We did not calculate the sample size with statistical rationale because we used  
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50  
51 248 a 3 + 3 dose-escalation design to confirm the safety and tolerability of CL2020.  
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54 249 The scheduled number of enrolled patients is 12.  
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6 251 **Statistical analysis**  
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9 252 All analyses are based on an intention-to-treat principle. All adverse events will  
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11  
12 253 be confirmed for the primary endpoint, and the proportions of the adverse  
13  
14  
15 254 events and their 95% CI based on the Clopper-Pearson method will be  
16  
17  
18 255 calculated. Time-to-event data will be summarized using the Kaplan-Meier  
19  
20  
21 256 method. Descriptive statistics for continuous variables and frequency and  
22  
23  
24 257 proportion for categorical variables will be calculated for each secondary  
25  
26  
27 258 endpoint. Statistical analysis will be performed using SAS software (SAS  
28  
29  
30 259 Institute, version 9.4, North Carolina, USA). Statistical significance will be  
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32  
33 260 defined as  $p < 0.05$ .  
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39 262 **Monitoring and auditing**  
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42 263 The monitoring personnel will investigate the progress of this trial and confirm  
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44  
45 264 the adequacy of the research procedures, and the auditing personnel will check  
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47  
48 265 the quality of this trial independent of the investigators, in accordance with the  
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51 266 laws, regulations, study protocol, and standard operating procedures.  
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57 268 **Status of this trial**  
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6 269 The Ministry of Health, Labour and Welfare accepted this clinical trial  
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9 270 notification as a trial on a new cellular and tissue-based product in January  
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11  
12 271 2020. The registration of the first participant and the administration of CL2020  
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14  
15 272 were completed in March 2020. Three patients were enrolled into a low-dose  
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18 273 cohort, whereas six patients were allocated to a high-dose cohort as of July  
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21 274 2021. Patient recruitment was performed in Nagoya University Hospital from  
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23  
24 275 February 2020 to July 2021, and the study will be terminated in September  
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27 276 2023.

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## 32 33 278 **Ethics and dissemination**

### 34 35 36 279 **Ethics approval**

37  
38  
39 280 This study was approved by the Nagoya University Hospital Institutional Review  
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41  
42 281 Board (No. 312005) on November 13, 2019. This study will be conducted in  
43  
44  
45 282 accordance with the Declaration of Helsinki and Good Clinical Practice. The  
46  
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48 283 investigators must always obtain approval from the Institutional Review Board  
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50  
51 284 about any amendment to the protocol and provide the necessary reasons.

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### 55 56 57 286 **Patient consent for participation**

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6 287 The investigators and trained clinical research coordinators will introduce the  
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9 288 trial to patients' legal representatives with prepared information sheets and  
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12 289 informed consent forms. The investigator will obtain written consent to  
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14  
15 290 participate in the trial. Identification of all subjects during the data collection will  
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18 291 be performed using a subject identification code, and all personnel involved in  
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21 292 this study will take the best possible precautions to ensure the protection of  
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24 293 patients' personal information.  
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### 295 **Dissemination**

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

298

### 299 **Discussion**

300 This clinical trial is aimed at evaluating the safety and tolerability of CL2020, a  
301 Muse cell-based product, in neonates. When CL2020 was administered  
302 intravenously to infant rats, the cells were distributed mainly in the lungs  
303 immediately after administration; however, there was no change in respiratory  
304 condition or pathological evaluation. Based on non-clinical study data and

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6 305 ongoing clinical trials progress of CL2020, we decided to implement this clinical  
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9 306 trial to ensure safety in neonates.

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12 307 Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy.

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15 308 Several randomized control trials of hypothermia therapy for HIE have been

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18 309 conducted,[32–37] and hypothermia is currently the sole neuroprotective

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21 310 therapy. However, its effectiveness is insufficient; therefore, a novel therapy is

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24 311 required. Regenerative therapy is the focus of next-generation therapy. Clinical

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27 312 studies with autologous UCBCs for HIE had been conducted before the

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30 313 development of CL2020.[10, 11] This UCBCs therapy requires additional

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33 314 equipment and human resources for its preparation because the newborns'

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36 315 umbilical cord blood has to be collected at birth, and the patients receive the

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39 316 first dose of prepared UCBCs within 24 hours after birth. In contrast, in our non-

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42 317 clinical study, single intravenous administration of Muse cells for HIE model rats

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44  
45 318 3 days after hypoxic-ischemic injury ameliorated behavioral abnormalities up to

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48 319 5 months.[22] In a non-clinical study using CL2020, the treatment effect was

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51 320 exerted at even later administration timing. Thus, we set the administration of

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54 321 Muse cells to human neonates between 5 and 14 days after birth, which means

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57 322 that physicians and patients' families can afford the time to decide or prepare

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6 323 the treatment based on patient condition or seek further opinions.  
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9 324 We conducted a consultation meeting about the main clinical trial design,  
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12 325 including the administration's timing as above with the Japanese regulatory  
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15 326 authority, Pharmaceutical and Medical Devices Agency, and they agreed to our  
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17  
18 327 proposed design of this trial. We will perform a randomized placebo-controlled  
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21 328 clinical trial to evaluate the effectiveness of CL2020 for HIE after confirming the  
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24 329 safety and tolerability of its intravenous administration in neonates.  
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27 330 Herein, we present the overall design of this single-center, open-label, dose-  
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30 331 escalation clinical trial of Muse cell products in HIE patients with hypothermia.  
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33 332 This clinical trial is the first clinical application of CL2020 in neonates based on  
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36 333 our non-clinical study results, and if we can verify the safety and well-tolerability  
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39 334 of this product in neonates, it may expand its application to other disorders in  
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42 335 neonates and children.  
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#### 48 337 **List of abbreviations**

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51 338 CI, confidence interval; DSMB, data and safety monitoring board; GMFCS:  
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54 339 expanded and revised gross motor function classification system; HIE, hypoxic-  
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57 340 ischemic encephalopathy; HLA, human leukocyte antigen; MSC, mesenchymal  
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6 341 stem cell; Muse cell, multilineage-differentiating stress-enduring cell; UCBC,

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9 342 umbilical cord blood cell

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15 344 **Authors' contributions**

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18 345 YS is the principal investigator in this trial and has access to all data. NM, SSh,

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20  
21 346 KU, TS, AK, MA, AH, and YS developed the study protocol. SSh and YS

22  
23  
24 347 participated in the concepts and design of the study. AK is a quality control

25  
26  
27 348 monitor, MA is responsible for data management, and AH supervised the

28  
29  
30 349 statistical analysis. NM, SSh, and MM supported preparation and management

31  
32  
33 350 of this study. KU, TS, SSu, RM, MH, and YS helped with the recruitment and

34  
35  
36 351 evaluation of patients, and prepared cells for administration. NM drafted and

37  
38  
39 352 revised the manuscript. SSh and YS have revised the manuscript. All authors

40  
41  
42 353 read and approved the final manuscript.

43  
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45 354

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48 355 **Acknowledgements**

49  
50  
51 356 The authors are grateful to LSII for providing the CL2020. We would like to

52  
53  
54 357 thank all the physicians who referred patients for this study and the staff at

55  
56  
57 358 Nagoya University Hospital for assisting with the recruitment and evaluation of

1  
2  
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5  
6 359 patients for this trial. We thank the DSMB members for evaluating the safety  
7  
8  
9 360 data in this study. We would like to thank Editage (www.editage.com) for  
10  
11  
12 361 English language editing.  
13  
14

15 362

### 18 363 **Funding statement**

21 364 This work was supported by the Japan Agency for Medical Research  
22  
23  
24 365 Development [grant number: JP21Im0203143].  
25  
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27 366

### 30 367 **Competing interests statement**

33 368 SSh, MM, and YS have collaborative projects with research funding from LSII  
34  
35  
36 369 for perinatal diseases. SSh and AH receive fees based on a consultation  
37  
38  
39 370 contract from LSII. SSh, TS, MM, MH, and YS have a patent for the application  
40  
41  
42 371 of Muse cells in the treatment of HIE and other indications. LSII provided  
43  
44  
45 372 CL2020 for this clinical trial free of charge.  
46  
47

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### 51 374 **Data availability statement**

54 375 The datasets generated and analyzed during this study will not be publicly  
55  
56  
57 376 available due to the confidentiality clause in the informed consent form.  
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378 **References**

- 379 1. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal  
380 encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*  
381 2010;86:329–38.
- 382 2. Hayakawa M, Ito Y, Saito S, et al. Incidence and prediction of outcome in  
383 hypoxic-ischemic encephalopathy in Japan. *Pediatr Int* 2014;56:215–21.
- 384 3. Lawn JE, Cousens S, Zupan J, et al. 4 million neonatal deaths: When?  
385 Where? Why? *Lancet* 2005;365:891–900.
- 386 4. Shankaran S. Therapeutic hypothermia for neonatal encephalopathy. *Curr*  
387 *Treat Options Neurol* 2012;14:608–19.
- 388 5. Perlman JM, Wyllie J, Kattwinkel J, et al. Neonatal resuscitation: 2010  
389 international consensus on cardiopulmonary resuscitation and emergency  
390 cardiovascular care science with treatment recommendations. *Circulation*  
391 2010;122 Suppl 1:S516–38.
- 392 6. Edwards AD, Brocklehurst P, Gunn AJ, et al. Neurological outcomes at 18  
393 months of age after moderate hypothermia for perinatal hypoxic ischaemic

- 1  
2  
3  
4  
5  
6 394       encephalopathy: synthesis and meta-analysis of trial data. *BMJ*  
7  
8  
9 395       2010;340:c363.  
10  
11  
12 396       7. Hattori T, Sato Y, Kondo T, et al. Administration of umbilical cord blood cells  
13  
14  
15 397       transiently decreased hypoxic-ischemic brain injury in neonatal rats. *Dev*  
16  
17  
18 398       *Neurosci* 2015;37:95–104.  
19  
20  
21 399       8. Tsuji M, Taguchi A, Ohshima M, et al. Effects of intravenous administration  
22  
23  
24 400       of umbilical cord blood CD34+ cells in a mouse model of neonatal stroke.  
25  
26  
27 401       *Neuroscience* 2014;263:148–58.  
28  
29  
30 402       9. Nakanishi K, Sato Y, Mizutani Y, et al. Rat umbilical cord blood cells  
31  
32  
33 403       attenuate hypoxic-ischemic brain injury in neonatal rats. *Sci Rep*  
34  
35  
36 404       2017;7:44111.  
37  
38  
39 405       10. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord  
40  
41  
42 406       blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr*  
43  
44  
45 407       2014;164:973–9.  
46  
47  
48 408       11. Tsuji M, Sawada M, Watabe S, et al. Autologous cord blood cell therapy for  
49  
50  
51 409       neonatal hypoxic-ischaemic encephalopathy: a pilot study for feasibility and  
52  
53  
54 410       safety. *Sci Rep* 2020;10:4603.  
55  
56  
57  
58  
59  
60

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2  
3  
4  
5  
6  
7 411 12. Mikrogeorgiou A, Sato Y, Kondo T, et al. Dedifferentiated fat cells as a  
8  
9 412 novel source for cell therapy to target neonatal hypoxic-ischemic  
10  
11  
12 413 encephalopathy. *Dev Neurosci* 2017;39:273–86.  
13  
14  
15 414 13. Sato Y, Ueda K, Kondo T, et al. Administration of bone marrow-derived  
16  
17  
18 415 mononuclear cells contributed to the reduction of hypoxic-ischemic brain  
19  
20  
21 416 injury in neonatal rats. *Front Neurol* 2018;9:987.  
22  
23  
24 417 14. Sugiyama Y, Sato Y, Kitase Y, et al. Intravenous administration of bone  
25  
26  
27 418 marrow-derived mesenchymal stem cell, but not adipose tissue-derived  
28  
29  
30 419 stem cell, ameliorated the neonatal hypoxic-ischemic brain injury by  
31  
32  
33 420 changing cerebral inflammatory state in rat. *Front Neurol* 2018;9:757.  
34  
35  
36 421 15. Kitase Y, Sato Y, Kazuto U, et al. A novel treatment with stem cells from  
37  
38  
39 422 human exfoliated deciduous teeth for hypoxic-ischemic encephalopathy in  
40  
41  
42 423 neonatal rats. *Stem Cells Dev* 2020;29:63–74.  
43  
44  
45 424 16. Kuroda Y, Kitada M, Wakao S, et al. Unique multipotent cells in adult  
46  
47  
48 425 human mesenchymal cell populations. *Proc Natl Acad Sci* 2010;107:8639–  
49  
50  
51 426 43.  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
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3  
4  
5  
6  
7 427 17. Dezawa M. Muse cells provide the pluripotency of mesenchymal stem  
8  
9 428 cells: direct contribution of Muse cells to tissue regeneration. *Cell*  
10  
11  
12 429 *Transplant* 2016;25:849–61.  
13  
14  
15 430 18. Wakao S, Akashi H, Kushida Y, et al. Muse cells, newly found non-  
16  
17  
18 431 tumorigenic pluripotent stem cells, reside in human mesenchymal tissues.  
19  
20  
21 432 *Pathol Int* 2014;64:1–9.  
22  
23  
24 433 19. Yamada Y, Wakao S, Kushida Y, et al. S1P–S1PR2 axis mediates homing  
25  
26  
27 434 of Muse cells into damaged heart for long-lasting tissue repair and  
28  
29  
30 435 functional recovery after acute myocardial infarction. *Circ Res*  
31  
32  
33 436 2018;122:1069–83.  
34  
35  
36 437 20. Wakao S, Kuroda Y, Ogura F, et al. Regenerative effects of mesenchymal  
37  
38  
39 438 stem cells: contribution of Muse cells, a novel pluripotent stem cell type that  
40  
41  
42 439 resides in mesenchymal cells. *Cells* 2012;1:1045–60.  
43  
44  
45 440 21. Dezawa M. Muse cells. Tokyo: Springer Japan 2018.  
46  
47  
48 441 22. Suzuki T, Sato Y, Kushida Y, et al. Intravenously delivered multilineage-  
49  
50  
51 442 differentiating stress enduring cells dampen excessive glutamate  
52  
53  
54 443 metabolism and microglial activation in experimental perinatal hypoxic  
55  
56  
57 444 ischemic encephalopathy. *J Cerebral Blood Flow Metab* 2021;41:1707–20.  
58  
59  
60

- 1  
2  
3  
4  
5  
6 445 23. Noda T, Nishigaki K, Minatoguchi S. Safety and efficacy of human Muse  
7  
8  
9 446 cell-based product for acute myocardial infarction in a first-in-human trial.  
10  
11  
12 447 *Circ J* 2020;84:1189–92.  
13  
14  
15 448 24. Fujita Y, Nohara T, Takashima S, et al. Intravenous allogeneic  
16  
17  
18 449 multilineage-differentiating stress-enduring cells in adults with dystrophic  
19  
20  
21 450 epidermolysis bullosa: a phase 1/2 open-label study. *J Eur Acad*  
22  
23  
24 451 *Dermatology Venerol* 2021;35:e528–31.  
25  
26  
27 452 25. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress.  
28  
29  
30 453 *Arch Neurol* 1976;33:696–705.  
31  
32  
33 454 26. Abe T, Aburakawa D, Niizuma K, et al. Intravenously transplanted human  
34  
35  
36 455 multilineage-differentiating stress-enduring cells afford brain repair in a  
37  
38  
39 456 mouse lacunar stroke model. *Stroke* 2020;51:601–11.  
40  
41  
42 457 27. Bayley N. Bayley scales of infant and toddler development. 3rd ed. San  
43  
44  
45 458 Antonio, TX: Psychological Corp 2006.  
46  
47  
48 459 28. Society for the Kyoto Scale of Psychological Development. The Kyoto  
49  
50  
51 460 scale of psychological development 2001: information for standardization  
52  
53  
54 461 and administration. Kyoto, Japan: Kyoto Kokusai Shakai Fukushi Center  
55  
56  
57 462 2002 [in Japanese].  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7 463 29. Fisher RS, Acevedo C, Arzimanoglou A, et al. A practical clinical definition  
8  
9 464 of epilepsy. *Epilepsia* 2014;55:475–82.
- 10  
11  
12 465 30. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor  
13  
14  
15 466 outcome in perinatal asphyxia: evaluation of MR scoring systems. *Am J*  
16  
17  
18 467 *Neuroradiol* 1998;19:143–9.
- 19  
20  
21 468 31. Palisano RJ, Rosenbaum P, Bartlett D, et al. Content validity of the  
22  
23  
24 469 expanded and revised Gross Motor Function Classification System. *Dev*  
25  
26  
27 470 *Med Child Neurol* 2008;50:744–50.
- 28  
29  
30 471 32. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to  
31  
32  
33 472 treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361:1349-58.
- 34  
35  
36 473 33. Jacobs SE, Morley CJ, Inder TE, et al. Whole-body hypothermia for term  
37  
38  
39 474 and near-term newborns with hypoxic-ischemic encephalopathy. *Arch*  
40  
41  
42 475 *Pediatr Adolesc Med* 2011;165:692-700.
- 43  
44  
45 476 34. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with  
46  
47  
48 477 mild systemic hypothermia after neonatal encephalopathy: Multicentre  
49  
50  
51 478 randomised trial. *Lancet* 2005;365:663-70.
- 52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3  
4  
5  
6  
7 479 35. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia  
8  
9 480 for neonates with hypoxic–ischemic encephalopathy. *N Engl J Med*  
10  
11  
12 481 2005;353:1574-84.  
13  
14  
15 482 36. Simbruner G, Mittal RA, Rohlmann F, et al. Systemic hypothermia after  
16  
17  
18 483 neonatal encephalopathy: outcomes of neo.nEURO.network RCT.  
19  
20  
21 484 *Pediatrics* 2010;126:e771–8.  
22  
23  
24 485 37. Zhou W, Cheng G, Shao X, et al. Selective head cooling with mild systemic  
25  
26  
27 486 hypothermia after neonatal hypoxic-ischemic encephalopathy: A multicenter  
28  
29  
30 487 randomized controlled trial in China. *J Pediatr* 2010;157:367-72.  
31  
32  
33  
34  
35  
36  
37  
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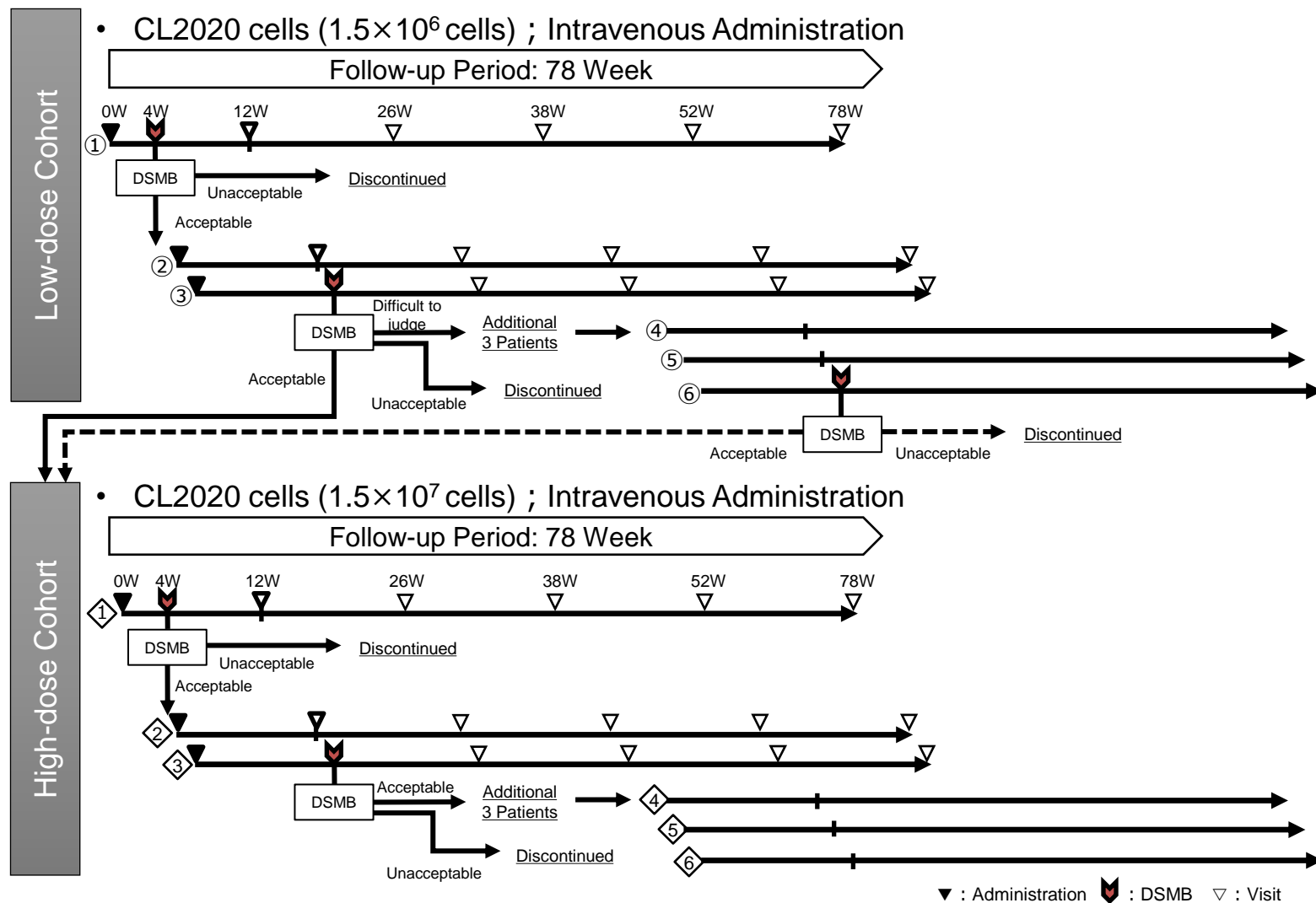


Figure 1 Study framework

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

		Reporting Item	Page Number
<b>Administrative information</b>			
Title	<a href="#">#1</a>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered, name of intended registry	3, 4
Trial registration: data set	<a href="#">#2b</a>	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	<a href="#">#3</a>	Date and version identifier	2
Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	23, 24
Roles and responsibilities: contributorship	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1, 22, 23

1	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1,2
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	n/a
9	responsibilities:		design; collection, management, analysis, and	
10	sponsor and funder		interpretation of data; writing of the report; and the	
11			decision to submit the report for publication,	
12			including whether they will have ultimate authority	
13			over any of these activities	
14				
15				
16				
17	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	12
18	responsibilities:		coordinating centre, steering committee, endpoint	
19	committees		adjudication committee, data management team,	
20			and other individuals or groups overseeing the trial,	
21			if applicable (see Item 21a for data monitoring	
22			committee)	
23				
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27	<b>Introduction</b>			
28				
29				
30	Background and	<a href="#">#6a</a>	Description of research question and justification for	5
31	rationale		undertaking the trial, including summary of relevant	
32			studies (published and unpublished) examining	
33			benefits and harms for each intervention	
34				
35				
36	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	n/a; This is a
37	rationale: choice of			single-arm,
38	comparators			dose-
39				escalation
40				trial.
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45	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	9
46				
47	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg,	9
48			parallel group, crossover, factorial, single group),	
49			allocation ratio, and framework (eg, superiority,	
50			equivalence, non-inferiority, exploratory)	
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54	<b>Methods:</b>			
55	<b>Participants,</b>			
56	<b>interventions, and</b>			
57	<b>outcomes</b>			
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1	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
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7				
8	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, 11
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14	Interventions: description	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
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20	Interventions: modifications	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
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27	Interventions: adherence	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
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32	Interventions: concomitant care	<a href="#">#11d</a>	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
33				
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36	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16
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48	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1
49				
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55	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study objectives and how it was determined,	17
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including clinical and statistical assumptions supporting any sample size calculations

Recruitment [#15](#) Strategies for achieving adequate participant enrolment to reach target sample size 9, 10

## Methods:

### Assignment of interventions (for controlled trials)

Allocation: sequence generation [#16a](#) Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions n/a; This study will be open label.

Allocation concealment mechanism [#16b](#) Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned n/a; This study will be open label.

Allocation: implementation [#16c](#) Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions n/a; This study will be open label.

Blinding (masking) [#17a](#) Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how n/a; This study will be open label.

Blinding (masking): emergency unblinding [#17b](#) If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a; This study will be open label.

### Methods: Data collection, management, and analysis

Data collection plan [#18a](#) Plans for assessment and collection of outcome, baseline, and other trial data, including any related 14

processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol

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10	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete
11	retention		follow-up, including list of any outcome data to be
12			collected for participants who discontinue or deviate
13			from intervention protocols
14			
15			
16			
17	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,
18			including any related processes to promote data
19			quality (eg, double data entry; range checks for data
20			values). Reference to where details of data
21			management procedures can be found, if not in the
22			protocol
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25			
26	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and
27			secondary outcomes. Reference to where other
28			details of the statistical analysis plan can be found, if
29			not in the protocol
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32			
33	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup
34	analyses		and adjusted analyses)
35			
36			
37	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol
38	population and		non-adherence (eg, as randomised analysis), and
39	missing data		any statistical methods to handle missing data (eg,
40			multiple imputation)
41			
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44	<b>Methods:</b>		
45	<b>Monitoring</b>		
46			
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48	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC);
49	formal committee		summary of its role and reporting structure;
50			statement of whether it is independent from the
51			sponsor and competing interests; and reference to
52			where further details about its charter can be found,
53			if not in the protocol. Alternatively, an explanation of
54			why a DMC is not needed
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1	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping	n/a
2	interim analysis		guidelines, including who will have access to these	
3			interim results and make the final decision to	
4			terminate the trial	
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8	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and	12,16
9			managing solicited and spontaneously reported	
10			adverse events and other unintended effects of trial	
11			interventions or trial conduct	
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14	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct,	18
15			if any, and whether the process will be independent	
16			from investigators and the sponsor	
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20	<b>Ethics and</b>			
21	<b>dissemination</b>			
22				
23				
24	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /	19
25	approval		institutional review board (REC / IRB) approval	
26				
27				
28	Protocol	<a href="#">#25</a>	Plans for communicating important protocol	19
29	amendments		modifications (eg, changes to eligibility criteria,	
30			outcomes, analyses) to relevant parties (eg,	
31			investigators, REC / IRBs, trial participants, trial	
32			registries, journals, regulators)	
33				
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36	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	19, 20
37			potential trial participants or authorised surrogates,	
38			and how (see Item 32)	
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41	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use	n/a
42	ancillary studies		of participant data and biological specimens in	
43			ancillary studies, if applicable	
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47	Confidentiality	<a href="#">#27</a>	How personal information about potential and	20
48			enrolled participants will be collected, shared, and	
49			maintained in order to protect confidentiality before,	
50			during, and after the trial	
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53	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	24
54	interests		investigators for the overall trial and each study site	
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1	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
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6	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
7	trial care			
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11	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20, 24
12	trial results			
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21	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of professional writers	n/a;
22	authorship			Authorship
23				eligibilities
24				were
25				confirmed by
26				standard
27				material.
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31				
32	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
33	reproducible			
34	research			
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38	<b>Appendices</b>			
39				
40	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given to participants and authorised surrogates	n/a
41	materials			
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45	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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# BMJ Open

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

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

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28 **Word count 2,600 words**

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35 **Abstract**

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

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

51 **Ethics and dissemination:** This study will be conducted in accordance with the

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7 52 Declaration of Helsinki and Good Clinical Practice. The Nagoya University Hospital  
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9 53 Institutional Review Board (No. 312005) approved this study on 13 November 2019.

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12 54 **Trial registration:** ClinicalTrials.gov: NCT04261335, registered on 7 February 2020,  
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14  
15 55 <https://clinicaltrials.gov/ct2/show/NCT04261335>. Japan Registry of Clinical Trials:  
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18 56 jRCT2043190112, registered on 6 February 2020, [https://jrct.niph.go.jp/latest-](https://jrct.niph.go.jp/latest-detail/jRCT2043190112)  
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21 57 [detail/jRCT2043190112](https://jrct.niph.go.jp/latest-detail/jRCT2043190112)

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### 26 27 59 **Strengths and limitations of this study**

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30 60 • This is the first clinical trial aimed at the safety and tolerability of CL2020, a Muse  
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33 61 cell-based product, in neonates.

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36 62 • Hypothermia is currently the sole neuroprotective therapy for HIE; its effectiveness  
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39 63 is insufficient, and a novel therapy is required.

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42 64 • After confirming the safety and tolerability of intravenous CL2020 in neonates, we  
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45 65 will need a randomised placebo-controlled clinical trial to evaluate the effectiveness  
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48 66 of CL2020 for treating HIE.

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51 67 • This trial is the first clinical application in neonates based on our non-clinical study  
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53  
54 68 results; if this product is found to be safe and well-tolerated by neonates, its

69 application may expand to other disorders in neonates and children.

## 70 **Keywords**

71 Hypoxic-ischaemic encephalopathy, neonates, cerebral palsy, hypothermia,  
72 mesenchymal stem cell, Muse cell

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## 75 **INTRODUCTION**

76 Neonatal hypoxic-ischaemic encephalopathy (HIE) results from acute perinatal  
77 asphyxia and can lead to poor patient outcomes, including death, physical disabilities,  
78 and mental retardation. HIE has an estimated incidence of 1.5 per 1,000 live births  
79 (95% confidence intervals [CI]: 1.3 to 1.7) from the three population-based studies  
80 in United Kingdom, Australia, Sweden carried out since 1980,[1] and the incidence  
81 of moderate or severe HIE has been reported to be 0.37 per 1,000 term live births in  
82 Japan.[2] Birth asphyxia accounts for 23% of global neonatal deaths.[3] Because  
83 HIE is associated with irreversible injury to the central nervous system, its sequelae  
84 such as cerebral palsy, epilepsy, or cognitive impairment could be major persistent  
85 burdens on both patients and their families.



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7 86 The most evidence-based treatment for moderate-to-severe HIE is therapeutic  
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10 87 hypothermia, which maintains a body temperature of 33°C–34°C for 72 hours[4, 5].  
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12 88 However, its effectiveness is limited. A previous study reported that the number  
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15 89 needed to treat was 9 (95% CI: 5–25) for hypothermia therapy to avoid 1 death or  
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18 90 severe disability at 18 months.[6] Therefore, a novel treatment for moderate-to-  
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21 91 severe HIE is warranted.

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24 92 Regenerative medicine has been developed as a new and effective treatment for  
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27 93 HIE. Preclinical animal studies using umbilical cord blood cells (UCBCs) in neonatal  
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30 94 HIE and stroke rat models have reported effectiveness.[7-9] In addition, some  
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33 95 exploratory clinical studies have shown the safety and feasibility of autologous  
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36 96 UCBCs administration for HIE neonates.[10, 11] However, preparing autologous  
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39 97 UCBCs requires well-equipped facilities and sufficient human resources in birthing  
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42 98 centres, clinics, or hospitals.

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45 99 From a wide variety of options as candidates for regenerative cells,[12-15] we have  
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48 100 noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells  
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51 101 are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent  
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54 102 markers that self-renew and differentiate from a single cell into each of the three  
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6 103 germ layer cells.[16] They are positive for stage-specific embryonic antigen (SSEA)-  
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9 104 3 and CD105 in the peripheral blood, bone marrow, and organ connective tissues.[17,  
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12 105 18] Muse cells also have a specific immunomodulatory system, represented by  
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15 106 human leukocyte antigen (HLA) -G expression, allowing them to be directly  
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18 107 administered without HLA matching or immunosuppressant agents.[19] Furthermore,  
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21 108 after intravenous administration, Muse cells are distributed to the damaged site by  
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24 109 sphingosine monophosphate (S1P)-sphingosine monophosphate receptor 2  
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27 110 (S1PR2) axis mechanism,[19] and then self-renewed without artificial differentiation  
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29  
30 111 or induction. After migrating, Muse cells differentiate into tissue-compatible cells  
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33 112 according to the microenvironment and remain integrated into the host tissue to  
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36 113 participate in tissue repair.[20, 21] Based on these characteristics, intravenous  
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39 114 administration of allogenic Muse cells is expected to be an effective regenerative  
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42 115 therapy for HIE.  
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45 116 We found that the systemic administration of human Muse cells in the perinatal HIE  
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48 117 rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left  
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51 118 carotid artery, improved learning deficits and motor impairment. In addition, human  
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54 119 Muse cells are localised in the damaged brain and differentiate into neurons.  
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6 120 These effects were much clearer in the Muse cells than in mesenchymal stem cells  
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9 121 (MSCs) without Muse cells subpopulation.[22] Moreover, we confirmed that the  
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12 122 human allogenic Muse cells-based product, CL2020, manufactured by Life Science  
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15 123 Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical  
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18 124 Holdings Corporation, exerted a therapeutic effect with no toxicity in the HIE rat  
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21 125 models. To verify the safety and effectiveness of CL2020, LSII has conducted  
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24 126 several clinical trials in adult patients with acute myocardial infarction (JapicCTI-  
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27 127 No.: JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103),  
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30 128 epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841),  
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33 129 amyotrophic lateral sclerosis (jRCT2063200047), and acute respiratory distress  
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36 130 syndrome associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-  
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39 131 human clinical trial for acute myocardial infarction was performed in 3 patients and  
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42 132 indicated that CL2020 was safe and significantly improved the left ventricular  
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45 133 ejection fraction.[23] A phase 1/2 open-label trial on adult epidermolysis bullosa  
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48 134 was also recently published. A total of 5 patients received a single injection of  
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51 135 CL2020, and the ulcer size was significantly reduced for up to 3 months.[24]  
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54 136 Nevertheless, the safety and tolerability of Muse cells in neonates are unknown  
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7 137 because they have never been administered to neonates. Based on these results,  
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9 138 we planned the first-in-neonate clinical trial to confirm the safety and tolerability of  
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12 139 CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy.  
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15 140 Hence, we describe the detailed design of an investigator-initiated clinical trial on  
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18 141 neonatal HIE to investigate the safety, tolerability, and efficacy in  
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21 142 neurodevelopmental outcomes at 18 months. This clinical trial is named “The  
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24 143 Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring  
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27 144 cell-based product in Neonatal Hypoxic-Ischemic Encephalopathy Patients with  
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30 145 Therapeutic Hypothermia in the Dose Escalation Clinical Trial” (the SHIELD trial).  
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## 33 34 35 147 **METHODS AND ANALYSIS**

### 36 37 38 148 **Objective and study design**

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41 149 The SHIELD trial’s main objective is to confirm the safety and tolerability of  
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44 150 intravenous CL2020 in neonates with HIE. This trial is a single-centre, open-label,  
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47 151 non-randomised, dose-escalation exploratory clinical trial. We have planned a  
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50 152 standard 3 + 3 dose-escalation design to examine the optimal dose of CL2020 for  
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53 153 neonatal safety and tolerability. The follow-up period is up to 78 weeks after  
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6 154 administering CL2020 to each patient.

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12 156 **Recruitment and setting**

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15 157 Patient recruitment is done in Nagoya University Hospital or by receiving referrals of  
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18 158 patients from other hospitals in our district. The investigators will obtain written  
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21 159 informed consent from the patients' legal parental authority before screening. After  
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24 160 screening and verifying the patients' eligibility, they will be registered for the trial.

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30 162 **Participants**

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33 163 We will recruit a maximum of 12 neonates with HIE who have received therapeutic  
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36 164 hypothermia. They must meet the following inclusion criteria:

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39 165 1) At least 36 weeks gestational age, and one of the following criteria (i–iii)

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42 166 i. Apgar score  $\leq 5$  at 10 minutes

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45 167 ii. Continued neonatal resuscitation for at least 10 minutes

46  
47  
48 168 iii. pH  $< 7.0$ , or base deficit  $\geq 16$  mmol/L in any blood sample obtained within  
49  
50  
51 169 60 min after birth

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53  
54 170 2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]

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6 171 3) Therapeutic hypothermia initiated within 6 hours after birth and continued for 72

8  
9 172 hours

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11  
12 173 4) Birth weight  $\geq 1,800$  g

13  
14  
15 174 5) Heart rate  $\geq 100$ /min, and SpO<sub>2</sub>  $\geq 90\%$

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17  
18 175 6) Able to provide voluntary informed consent after receiving information about the

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20  
21 176 study (consent will be obtained from a legal proxy).

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23  
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26  
27 178 Exclusion criteria are:

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30 179 1) Suspected or confirmed severe congenital abnormalities or chromosomal

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32  
33 180 anomaly

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35  
36 181 2) Planned to undergo surgery or radiation therapy

37  
38  
39 182 3) Scheduled to take systemic corticosteroids treatment for over 5 days

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41  
42 183 4) Blood glucose  $\geq 200$  mg/dL continuously sustained

43  
44  
45 184 5) Participation in another interventional clinical study

46  
47  
48 185 6) Suspected or confirmed active and severe infection

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50  
51 186 7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or

52  
53  
54 187 syphilis serum reaction

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6 188 8) History of severe hypersensitivity or anaphylactic reaction

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8  
9 189 9) Severe complications not related to HIE

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12 190

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15 191 **Patient and public involvement**

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18 192 Patients' guardians or members of the public were not involved in this study protocol  
19  
20  
21 193 planning.

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23  
24 194 **Intervention and follow-up**

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26  
27 195 The clinical-grade Muse cell-based product, CL2020 ( $1.5 \times 10^7$  cells/15 mL of frozen  
28  
29  
30 196 preparation), was produced from human allogenic MSCs by LSII.[26] The CL2020  
31  
32  
33 197 was produced by exposing MSCs to some stressors, and they were enriched to be  
34  
35  
36 198 positive for both SSEA3 and CD105 but negative for CD45. We will prepare cells  
37  
38  
39 199 from CL2020 for administration to neonates by centrifuging the product after thawing,  
40  
41  
42 200 removing the supernatant, and suspending with acetated Ringer's solution as 15  
43  
44  
45 201 million cells in 15 mL. The patients will receive the prepared cells intravenously once  
46  
47  
48 202 between 5 and 14 days after birth. This study will utilise a 3 + 3 dose-escalation  
49  
50  
51 203 design, setting two cohorts for the injected dose. Subjects in the low-dose cohort will  
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53  
54 204 receive 1.5 million cells in 1.5 mL for 2 min, while those in the high-dose cohort will

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6 205 receive 15 million cells in 15 mL for 20 minutes. The following treatments will be  
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8  
9 206 prohibited during the study: corticosteroids (prednisolone converted at 2 mg/kg/day  
10  
11  
12 207 or more, and more than 5 days), other human MSC products, processed cell  
13  
14  
15 208 products except for the red blood cells, other investigational products, and the use  
16  
17  
18 209 of investigational medical devices. The data and safety monitoring board (DSMB)  
19  
20  
21 210 will consist of 3 specialists in paediatric and perinatal care independent of the trial  
22  
23  
24 211 investigators. The DSMB will be held at predefined times in both cohorts: at 4 weeks  
25  
26  
27 212 after administering to the first patient and 12 weeks after administering to the third  
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29  
30 213 patient in each cohort. The council will also be held when a product-related severe  
31  
32  
33 214 adverse event occurs or when investigators consider that it should be convened due  
34  
35  
36 215 to safety concerns. The DSMB will recommend whether this trial should be moved  
37  
38  
39 216 forward or be discontinued. Figure 1 illustrates the framework of this study. The study  
40  
41  
42 217 participants will be hospitalised for at least 2 weeks after CL2020 administration and  
43  
44  
45 218 followed up for 78 weeks. The planned visits and data collection are presented in  
46  
47  
48 219 Table 1.





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3			
4	Epilepsy		
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6	MRI	x	x
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8	Bayley scale <sup>e</sup>		x
9			
10	Kyoto scale <sup>f</sup>		x
11			
12	GMFCS <sup>g</sup>		x

<sup>a</sup>Blood pressure, pulse rate, and body temperature

<sup>b</sup>Red blood cell count, haemoglobin, haematocrit, white blood cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monocytes), and platelet count

<sup>c</sup>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.

<sup>d</sup>pH, urine protein, urine occult blood, and urine sugar

<sup>e</sup>Bayley Scales of Infant and Toddler Development Third edition

<sup>f</sup>Kyoto Scale of Psychological Development 2001

<sup>g</sup>Expanded and Revised Gross Motor Function Classification System

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6 **223 Study endpoints**  
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9 **224** The primary outcome is the incidence of adverse events until 12 weeks after  
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11  
12 **225** administration. The secondary outcomes are as follows:  
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15 **226** 1) Incidence of composite endpoints (death, continuous respiratory support, or  
16  
17  
18 **227** continuous use of vasopressors or pulmonary vasodilators)  
19

20  
21 **228** 2) Mortality and overall survival  
22

23  
24 **229** 3) Duration of continuous respiratory support: The definition of respiratory  
25  
26  
27 **230** support is the status of conducting artificial ventilation with tracheal intubation.  
28

29  
30 **231** 4) Duration of continuous use of vasopressors or pulmonary vasodilators:  
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32  
33 **232** dopamine, dobutamine, adrenaline, noradrenaline, milrinone, vasopressin,  
34  
35  
36 **233** *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide,  
37  
38  
39 **234** epoprostenol sodium, nitroglycerin, and alprostadil alfadex  
40

41  
42 **235** 5) The Bayley Scales of Infant and Toddler Development Third Edition [27]  
43  
44  
45 **236** score at 78 weeks  
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48 **237** 6) Developmental quotient as per the Kyoto Scale of Psychological  
49  
50  
51 **238** Development 2001[28] at 78 weeks  
52

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54 **239** 7) Assessment of postnatal development such as head control, rolling, sitting,  
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56  
57 **240** crawling, unaided walking, and saying several meaningful words  
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6 241 8) Presence of spasticity: The definition of spasticity is the status of increased  
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9 242 muscle tone or increased deep tendon reflex.  
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12 243 9) Presence of epilepsy: The definition of epilepsy is based on the International  
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15 244 League Against Epilepsy.[29]  
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18 245 10) Magnetic resonance imaging score: The scoring system is based on the  
19  
20  
21 246 report of Barkovich et al.[30]  
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23  
24 247 11) The score of Expanded and Revised Gross Motor Function Classification  
25  
26  
27 248 System[31] at 78 weeks

29  
30 249 In addition, we will collect vital signs and laboratory values for safety assessment  
31  
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33 250 at specific points, as shown in Table 1.  
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36 251

### 39 252 **Sample size calculation**

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42 253 We did not calculate the sample size with statistical rationale because we used a  
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44  
45 254 3 + 3 dose-escalation design to confirm the safety and tolerability of CL2020. The  
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47  
48 255 scheduled number of enrolled patients is 12.  
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51 256

### 54 257 **Statistical analysis**

55  
56  
57 258 All analyses are based on an intention-to-treat principle. We will analyse adverse  
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6 259 events on the safety analysis set defined as all subjects enrolled in this study and  
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8  
9 260 received the investigational cell product. All adverse events will be confirmed for  
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11  
12 261 the primary endpoint, and the proportions of the adverse events and their 95% CI  
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14  
15 262 based on the Clopper-Pearson method will be calculated. Overall survival,  
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18 263 defined as the time from birth to the date of death due to any cause, will be  
19  
20  
21 264 summarised using the Kaplan-Meier method. Descriptive statistics for continuous  
22  
23  
24 265 variables and frequency and proportion for categorical variables will be calculated  
25  
26  
27 266 for each secondary endpoint. Statistical analysis will be performed using the SAS  
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29  
30 267 software (SAS Institute, version 9.4, North Carolina, USA). Statistical significance  
31  
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33 268 will be defined as  $p < 0.05$ .

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36 26937  
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39 270 **Monitoring and auditing**

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42 271 The monitoring personnel will investigate the progress of this trial and confirm the  
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45 272 adequacy of the research procedures. The auditing personnel will check the  
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48 273 quality of this trial independent of the investigators, according to the laws,  
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51 274 regulations, study protocol, and standard operating procedures.

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277 **Status of this trial**

278 The Ministry of Health, Labour and Welfare accepted this clinical trial notification  
279 as a trial on a new cellular and tissue-based product in January 2020. The first  
280 participant was registered, and CL2020 was administered in March 2020. Three  
281 patients were enrolled into a low-dose cohort, while six were allocated to a high-  
282 dose cohort as of July 2021. Patient recruitment was performed in Nagoya  
283 University Hospital from February 2020 to July 2021, and the study will be  
284 terminated in September 2023.

285

286 **ETHICS AND DISSEMINATION**

287 **Ethical approval**

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

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6 295 **Patient consent for participation**  
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9 296 The investigators and trained clinical research coordinators will introduce the trial  
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12 297 to patients' legal representatives with prepared information sheets and informed  
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14  
15 298 consent forms. The investigator will obtain written consent to participate in the  
16  
17  
18 299 trial. Subjects will be identified during the data collection using a subject  
19  
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21 300 identification code. All personnel involved in this study will take the best possible  
22  
23  
24 301 precautions to ensure the protection of patients' personal information.  
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27 302  
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30 303 **Dissemination**  
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32  
33 304 The results of this clinical trial will be published in peer-reviewed journals,  
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36 305 presented in conferences, and submitted to clinical trial registries.  
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42 307 **DISCUSSION**  
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45 308 This clinical trial aims to evaluate the safety and tolerability of CL2020, a Muse  
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48 309 cell-based product, in neonates. When CL2020 was administered intravenously  
49  
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51 310 to infant rats, the cells were distributed mainly in the lungs immediately after  
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54 311 administration. However, there was no change in respiratory condition or  
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57 312 pathological evaluation. Based on non-clinical study data and ongoing clinical  
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6 313 trials of CL2020, we decided to implement this clinical trial to ensure safety in  
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9 314 neonates.

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12 315 Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy.

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15 316 Several randomised controlled trials of hypothermia therapy for HIE have been  
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18 317 conducted,[32-37] and hypothermia is currently the sole neuroprotective therapy.

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21 318 However, its effectiveness is insufficient, and a novel therapy is required.

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23  
24 319 Regenerative therapy is the focus of next-generation therapy. Clinical studies

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27 320 with autologous UCBCs for HIE had been conducted before the development of

28  
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30 321 CL2020.[10, 11] This UCBCs therapy requires additional equipment and human

31  
32  
33 322 resources for its preparation because the newborns' umbilical cord blood has to

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36 323 be collected at birth, and the patients receive the first dose of prepared UCBCs

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39 324 within 24 hours after birth. In contrast, in our non-clinical study, single intravenous

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42 325 administration of Muse cells to HIE model rats 3 days after hypoxic-ischaemic

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45 326 injury ameliorated behavioural abnormalities up to 5 months.[22] In a non-clinical

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47  
48 327 study using CL2020, the treatment effect was exerted at even 7 days after insult

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50  
51 328 by hypoxic ischaemia. In addition, a single dose of CL2020 administered via the

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54 329 vein at the subacute (about 9 days after onset) and chronic phases (about 30

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57 330 days) was effective in a mouse lacunar stroke model.[26] Thus, we set the



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6 331 administration of Muse cells to human neonates between 5 and 14 days after  
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9 332 birth, which means that physicians and patients' families can afford the time to  
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12 333 decide or prepare the treatment based on the patient's condition or seek other  
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14  
15 334 opinions.

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18 335 We held a consultation meeting about the main clinical trial design, including the  
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21 336 timing of administration as above with the Japanese regulatory authority,  
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24 337 Pharmaceutical and Medical Devices Agency, and they agreed to our proposed  
25  
26  
27 338 design for this trial. We will perform a randomised placebo-controlled clinical trial  
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29  
30 339 to evaluate the effectiveness of CL2020 for HIE after confirming the safety and  
31  
32  
33 340 tolerability of its intravenous administration in neonates.

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36 341 Herein, we present the overall design of this single-centre, open-label, dose-  
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39 342 escalation clinical trial of Muse cell products in HIE patients with hypothermia.  
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41  
42 343 This clinical trial is the first clinical application of CL2020 in neonates based on  
43  
44  
45 344 our non-clinical study results. If we can verify that this product is safe and well-  
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48 345 tolerable in neonates, its application may expand to other disorders in neonates  
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51 346 and children.

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6 349 **Funding**

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9 350 This work was supported by the Japan Agency for Medical Research  
10  
11  
12 351 Development [grant number: JP21Im0203143].  
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18 353 **References**

- 19  
20  
21 354 1 Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal  
22  
23  
24 355 encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*  
25  
26  
27 356 2010;86:329–38.  
28  
29  
30 357 2 Hayakawa M, Ito Y, Saito S, et al. Incidence and prediction of outcome in  
31  
32  
33 358 hypoxic-ischemic encephalopathy in Japan. *Pediatr Int* 2014;56:215–21.  
34  
35  
36 359 3 Lawn JE, Cousens S, Zupan J, et al. 4 million neonatal deaths: When? Where?  
37  
38  
39 360 Why? *Lancet* 2005;365:891–900.  
40  
41  
42 361 4 Shankaran S. Therapeutic hypothermia for neonatal encephalopathy. *Curr*  
43  
44  
45 362 *Treat Options Neurol* 2012;14:608–19.  
46  
47  
48 363 5 Perlman JM, Wyllie J, Kattwinkel J, et al. Part 11: Neonatal resuscitation: 2010  
49  
50  
51 364 international consensus on cardiopulmonary resuscitation and emergency  
52  
53  
54 365 cardiovascular care science with treatment recommendations. *Circulation*  
55  
56  
57 366 2010;122 Suppl 2:S516–38.  
58  
59  
60

- 1  
2  
3  
4  
5  
6 367 6 Edwards AD, Brocklehurst P, Gunn AJ, et al. Neurological outcomes at 18  
7  
8  
9 368 months of age after moderate hypothermia for perinatal hypoxic ischaemic  
10  
11  
12 369 encephalopathy: synthesis and meta-analysis of trial data. *BMJ*  
13  
14  
15 370 2010;340:c363.  
16  
17  
18 371 7 Hattori T, Sato Y, Kondo T, et al. Administration of umbilical cord blood cells  
19  
20  
21 372 transiently decreased hypoxic-ischemic brain injury in neonatal rats. *Dev*  
22  
23  
24 373 *Neurosci* 2015;37:95–104.  
25  
26  
27 374 8 Tsuji M, Taguchi A, Ohshima M, et al. Effects of intravenous administration of  
28  
29  
30 375 umbilical cord blood CD34(+) cells in a mouse model of neonatal stroke.  
31  
32  
33 376 *Neuroscience* 2014;263:148–58.  
34  
35  
36 377 9 Nakanishi K, Sato Y, Mizutani Y, et al. Rat umbilical cord blood cells attenuate  
37  
38  
39 378 hypoxic-ischemic brain injury in neonatal rats. *Sci Rep* 2017;7:44111.  
40  
41  
42 379 10 Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord  
43  
44  
45 380 blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr*  
46  
47  
48 381 2014;164:973–9.  
49  
50  
51 382 11 Tsuji M, Sawada M, Watabe S, et al. Autologous cord blood cell therapy for  
52  
53  
54 383 neonatal hypoxic-ischaemic encephalopathy: a pilot study for feasibility and  
55  
56  
57 384 safety. *Sci Rep* 2020;10:4603.  
58  
59  
60

- 1  
2  
3  
4  
5  
6  
7 385 12 Mikrogeorgiou A, Sato Y, Kondo T, et al. Dedifferentiated fat cells as a novel  
8  
9 386 source for cell therapy to target neonatal hypoxic-ischemic encephalopathy.  
10  
11  
12 387 *Dev Neurosci* 2017;39:273–86.
- 13  
14  
15 388 13 Sato Y, Ueda K, Kondo T, et al. Administration of bone marrow-derived  
16  
17  
18 389 mononuclear cells contributed to the reduction of hypoxic-ischemic brain  
19  
20  
21 390 injury in neonatal rats. *Front Neurol* 2018;9:987.
- 22  
23  
24 391 14 Sugiyama Y, Sato Y, Kitase Y, et al. Intravenous administration of bone  
25  
26  
27 392 marrow-derived mesenchymal stem cell, but not adipose tissue-derived stem  
28  
29  
30 393 cell, ameliorated the neonatal hypoxic-ischemic brain injury by changing  
31  
32  
33 394 cerebral inflammatory state in rat. *Front Neurol* 2018;9:757.
- 34  
35  
36 395 15 Kitase Y, Sato Y, Ueda K, et al. A novel treatment with stem cells from human  
37  
38  
39 396 exfoliated deciduous teeth for hypoxic-ischemic encephalopathy in neonatal  
40  
41  
42 397 rats. *Stem Cells Dev* 2020;29:63–74.
- 43  
44  
45 398 16 Kuroda Y, Kitada M, Wakao S, et al. Unique multipotent cells in adult human  
46  
47  
48 399 mesenchymal cell populations. *Proc Natl Acad Sci U S A* 2010;107:8639–43.
- 49  
50  
51 400 17 Dezawa M. Muse cells provide the pluripotency of mesenchymal stem cells:  
52  
53  
54 401 direct contribution of Muse cells to tissue regeneration. *Cell Transplant*  
55  
56  
57 402 2016;25:849–61.  
58  
59  
60

- 1  
2  
3  
4  
5  
6 403 18 Wakao S, Akashi H, Kushida Y, et al. Muse cells, newly found non-tumorigenic  
7  
8  
9 404 pluripotent stem cells, reside in human mesenchymal tissues. *Pathol Int*  
10  
11  
12 405 2014;64:1–9.  
13  
14  
15 406 19 Yamada Y, Wakao S, Kushida Y, et al. S1P–S1PR2 axis mediates homing of  
16  
17  
18 407 Muse cells into damaged heart for long-lasting tissue repair and functional  
19  
20  
21 408 recovery after acute myocardial infarction. *Circ Res* 2018;122:1069–83.  
22  
23  
24 409 20 Wakao S, Kuroda Y, Ogura F, et al. Regenerative effects of mesenchymal  
25  
26  
27 410 stem cells: contribution of Muse cells, a novel pluripotent stem cell type that  
28  
29  
30 411 resides in mesenchymal cells. *Cells* 2012;1:1045–60.  
31  
32  
33 412 21 Dezawa M. Clinical trials of muse cells. In: Dezawa M, eds. Muse cells.  
34  
35  
36 413 Advances in Experimental Medicine and Biology, vol 1103. Tokyo: Springer  
37  
38  
39 414 2018:305–7.  
40  
41  
42 415 22 Suzuki T, Sato Y, Kushida Y, et al. Intravenously delivered multilineage-  
43  
44  
45 416 differentiating stress enduring cells dampen excessive glutamate metabolism  
46  
47  
48 417 and microglial activation in experimental perinatal hypoxic ischemic  
49  
50  
51 418 encephalopathy. *J Cerebral Blood Flow Metab* 2021;41:1707–20.  
52  
53  
54  
55  
56  
57  
58  
59  
60

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2  
3  
4  
5  
6 419 23 Noda T, Nishigaki K, Minatoguchi S. Safety and efficacy of human Muse cell-  
7  
8  
9 420 based product for acute myocardial infarction in a first-in-human trial. *Circ J*  
10  
11  
12 421 2020;84:1189–92.  
13  
14  
15 422 24 Fujita Y, Nohara T, Takashima S, et al. Intravenous allogeneic multilineage-  
16  
17  
18 423 differentiating stress-enduring (Muse) cells in adults with dystrophic  
19  
20  
21 424 epidermolysis bullosa: a phase 1/2 open-label study. *J Eur Acad Dermatology*  
22  
23  
24 425 *Venerol* 2021;35:e528–31.  
25  
26  
27 426 25 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A  
28  
29  
30 427 clinical and electroencephalographic study. *Arch Neurol* 1976;33:696–705.  
31  
32  
33 428 26 Abe T, Aburakawa D, Niizuma K, et al. Intravenously transplanted human  
34  
35  
36 429 multilineage-differentiating stress-enduring cells afford brain repair in a  
37  
38  
39 430 mouse lacunar stroke model. *Stroke* 2020;51:601–11.  
40  
41  
42 431 27 Bayley N. Bayley scales of infant and toddler development. 3rd ed. San  
43  
44  
45 432 Antonio, TX: Psychological Corp 2006:  
46  
47  
48 433 28 Society for the Kyoto Scale of Psychological Development. The Kyoto scale  
49  
50  
51 434 of psychological development 2001: information for standardization and  
52  
53  
54 435 administration. Kyoto, Japan: Kyoto Kokusai Shakai Fukushi Center 2002 [in  
55  
56  
57 436 Japanese].  
58  
59  
60

- 1  
2  
3  
4  
5  
6 437 29 Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical  
7  
8  
9 438 clinical definition of epilepsy. *Epilepsia* 2014;55:475–82.  
10  
11  
12 439 30 Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome  
13  
14  
15 440 in perinatal asphyxia: evaluation of MR scoring systems. *Am J Neuroradiol*  
16  
17  
18 441 1998;19:143–9.  
19  
20  
21 442 31 Palisano RJ, Rosenbaum P, Bartlett D, et al. Content validity of the expanded  
22  
23  
24 443 and revised Gross Motor Function Classification System. *Dev Med Child*  
25  
26  
27 444 *Neurol* 2008;50:744–50.  
28  
29  
30 445 32 Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat  
31  
32  
33 446 perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361:1349–58.  
34  
35  
36 447 33 Jacobs SE, Morley CJ, Inder TE, et al. Whole-body hypothermia for term and  
37  
38  
39 448 near-term newborns with hypoxic-ischemic encephalopathy. *Arch Pediatr*  
40  
41  
42 449 *Adolesc Med* 2011;165:692–700.  
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45 450 34 Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild  
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48 451 systemic hypothermia after neonatal encephalopathy: multicentre  
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51 452 randomised trial. *Lancet* 2005;365:663–70.  
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6 453 35 Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for  
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9 454 neonates with hypoxic–ischemic encephalopathy. *N Engl J Med*  
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12 455 2005;353:1574–84.

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15 456 36 Simbruner G, Mittal RA, Rohlmann F, et al. Systemic hypothermia after  
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18 457 neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics*  
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21 458 2010;126:e771–8.

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24 459 37 Zhou W, Cheng G, Shao X, et al. Selective head cooling with mild systemic  
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27 460 hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter  
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30 461 randomized controlled trial in China. *J Pediatr* 2010;157:367–72.

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### 34 35 36 463 **Contributorship Statement**

37  
38  
39 464 YS is the principal investigator in this trial and has access to all data. NM, SSh,  
40  
41  
42 465 KU, TS, AK, MA, AH, and YS developed the study protocol. SSh and YS  
43  
44  
45 466 participated in the conception and design of the study. AK is a quality control  
46  
47  
48 467 monitor, MA is responsible for data management, and AH supervises the  
49  
50  
51 468 statistical analysis. NM, SSh, and MM supported the preparation and  
52  
53  
54 469 management of this study. KU, TS, SSu, RM, MH, and YS helped recruit and  
55  
56  
57 470 evaluate patients and prepare cells for administration. NM drafted and revised  
58  
59  
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6 471 the manuscript. SSh and YS have revised the manuscript. All authors read and  
7  
8  
9 472 approved the final manuscript.

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12 473

13  
14  
15 474 **Competing Interests**

16  
17  
18 475 SSh, MM, and YS have collaborative projects with research funding from LSII for  
19  
20  
21 476 perinatal diseases. SSh and AH receive fees based on a consultation contract  
22  
23  
24 477 from LSII. SSh, TS, MM, MH, and YS have a patent for the application of Muse  
25  
26  
27 478 cells in the treatment of HIE and other indications. LSII provided CL2020 for this  
28  
29  
30 479 clinical trial free of charge.

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39 482 **Acknowledgements**

40  
41  
42 483 The authors are grateful to LSII for providing the CL2020. We would like to thank  
43  
44  
45 484 all the physicians who referred patients for this study and the staff at Nagoya  
46  
47  
48 485 University Hospital for assisting with the recruitment and evaluation of patients  
49  
50  
51 486 for this trial. We thank the DSMB members for evaluating the safety data in this  
52  
53  
54 487 study. We would like to thank Editage ([www.editage.com](http://www.editage.com)) for English language  
55  
56  
57 488 editing.

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489 **Data availability statement**

490 The datasets generated and analysed during this study will not be publicly  
491 available due to the confidentiality clause in the informed consent form.

492

493 **Figure 1**

494 **Study framework**

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

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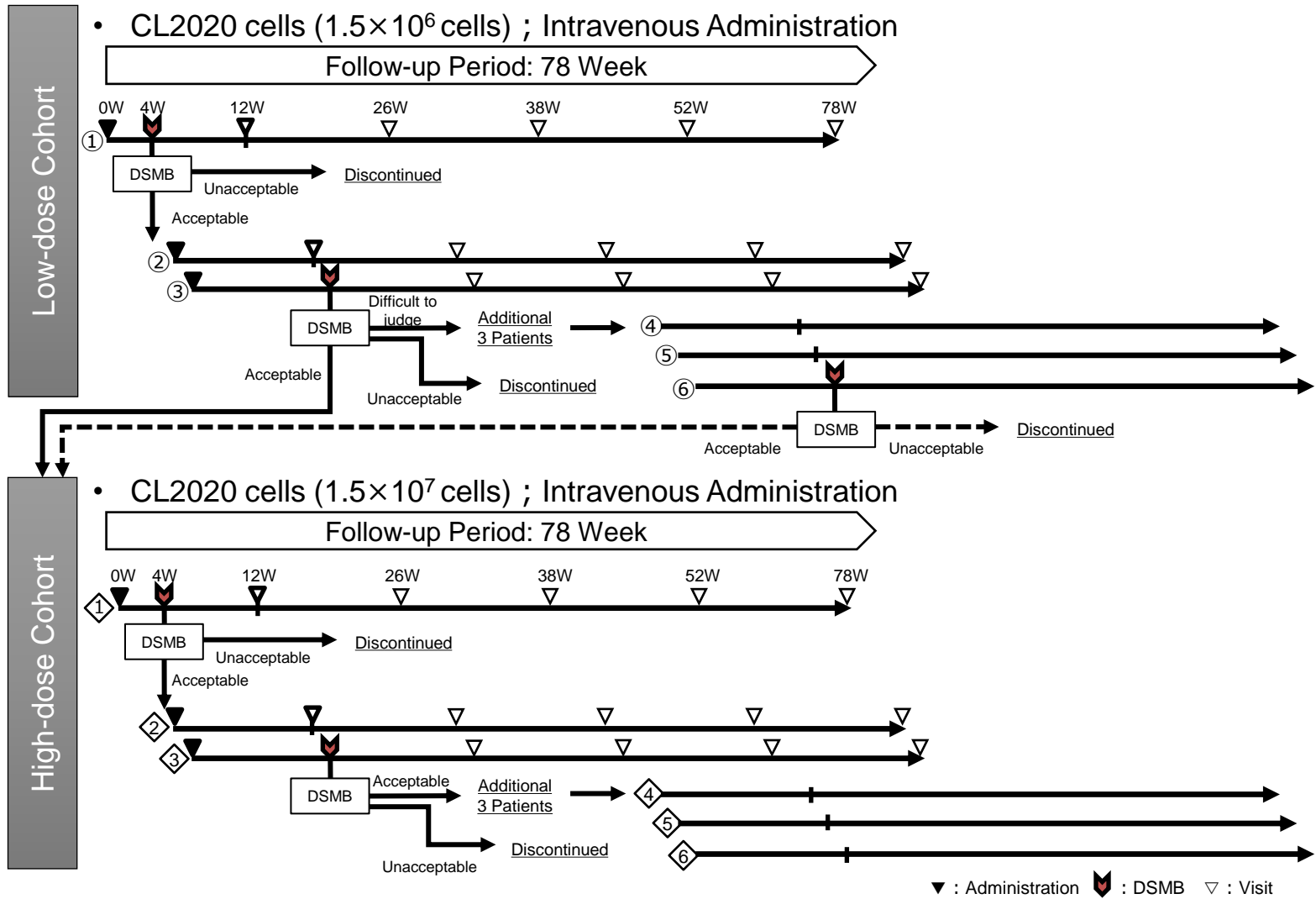


Figure 1 Study framework

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

		Reporting Item	Page Number
<b>Administrative information</b>			
Title	<a href="#">#1</a>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered, name of intended registry	3, 4
Trial registration: data set	<a href="#">#2b</a>	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	<a href="#">#3</a>	Date and version identifier	2
Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	23, 24
Roles and responsibilities: contributorship	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1, 22, 23

1	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1,2
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	n/a
9	responsibilities:		design; collection, management, analysis, and	
10	sponsor and funder		interpretation of data; writing of the report; and the	
11			decision to submit the report for publication,	
12			including whether they will have ultimate authority	
13			over any of these activities	
14				
15				
16				
17	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	12
18	responsibilities:		coordinating centre, steering committee, endpoint	
19	committees		adjudication committee, data management team,	
20			and other individuals or groups overseeing the trial,	
21			if applicable (see Item 21a for data monitoring	
22			committee)	
23				
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26				
27	<b>Introduction</b>			
28				
29				
30	Background and	<a href="#">#6a</a>	Description of research question and justification for	5
31	rationale		undertaking the trial, including summary of relevant	
32			studies (published and unpublished) examining	
33			benefits and harms for each intervention	
34				
35				
36	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	n/a; This is a
37	rationale: choice of			single-arm,
38	comparators			dose-
39				escalation
40				trial.
41				
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45	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	9
46				
47	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg,	9
48			parallel group, crossover, factorial, single group),	
49			allocation ratio, and framework (eg, superiority,	
50			equivalence, non-inferiority, exploratory)	
51				
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54	<b>Methods:</b>			
55	<b>Participants,</b>			
56	<b>interventions, and</b>			
57	<b>outcomes</b>			
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1	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
2				
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8	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, 11
9				
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14	Interventions: description	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
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20	Interventions: modifications	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
21				
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27	Interventions: adherence	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
28				
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32	Interventions: concomitant care	<a href="#">#11d</a>	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
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36	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16
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48	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1
49				
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55	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study objectives and how it was determined,	17
56				
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including clinical and statistical assumptions supporting any sample size calculations

Recruitment [#15](#) Strategies for achieving adequate participant enrolment to reach target sample size 9, 10

## Methods:

### Assignment of interventions (for controlled trials)

Allocation: sequence generation [#16a](#) Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions n/a; This study will be open label.

Allocation concealment mechanism [#16b](#) Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned n/a; This study will be open label.

Allocation: implementation [#16c](#) Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions n/a; This study will be open label.

Blinding (masking) [#17a](#) Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how n/a; This study will be open label.

Blinding (masking): emergency unblinding [#17b](#) If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a; This study will be open label.

### Methods: Data collection, management, and analysis

Data collection plan [#18a](#) Plans for assessment and collection of outcome, baseline, and other trial data, including any related 14

processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	Data collection plan: retention	<a href="#">#18b</a>	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
16 17 18 19 20 21 22 23 24 25	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
26 27 28 29 30 31 32	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
33 34 35 36	Statistics: additional analyses	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
37 38 39 40 41 42 43	Statistics: analysis population and missing data	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a

#### Methods: Monitoring

44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60	Data monitoring: formal committee	<a href="#">#21a</a>	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
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1	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping	n/a
2	interim analysis		guidelines, including who will have access to these	
3			interim results and make the final decision to	
4			terminate the trial	
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8	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and	12,16
9			managing solicited and spontaneously reported	
10			adverse events and other unintended effects of trial	
11			interventions or trial conduct	
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14	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct,	18
15			if any, and whether the process will be independent	
16			from investigators and the sponsor	
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20	<b>Ethics and</b>			
21	<b>dissemination</b>			
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24	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /	19
25	approval		institutional review board (REC / IRB) approval	
26				
27				
28	Protocol	<a href="#">#25</a>	Plans for communicating important protocol	19
29	amendments		modifications (eg, changes to eligibility criteria,	
30			outcomes, analyses) to relevant parties (eg,	
31			investigators, REC / IRBs, trial participants, trial	
32			registries, journals, regulators)	
33				
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35				
36	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	19, 20
37			potential trial participants or authorised surrogates,	
38			and how (see Item 32)	
39				
40				
41	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use	n/a
42	ancillary studies		of participant data and biological specimens in	
43			ancillary studies, if applicable	
44				
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46	Confidentiality	<a href="#">#27</a>	How personal information about potential and	20
47			enrolled participants will be collected, shared, and	
48			maintained in order to protect confidentiality before,	
49			during, and after the trial	
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53	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	24
54	interests		investigators for the overall trial and each study site	
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1	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
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6	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
7	trial care			
8				
9				
10				
11	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20, 24
12	trial results			
13				
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21	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of professional writers	n/a;
22	authorship			Authorship
23				eligibilities
24				were
25				confirmed by
26				standard
27				material.
28				
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31				
32	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
33	reproducible			
34	research			
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38	<b>Appendices</b>			
39				
40	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given to participants and authorised surrogates	n/a
41	materials			
42				
43				
44				
45	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-057073.R2
Article Type:	Protocol
Date Submitted by the Author:	24-Mar-2022
Complete List of Authors:	Matsuyama, Nao; Nagoya University Hospital, Department of Advanced Medicine Shimizu, Shinobu; Nagoya University Hospital, Department of Advanced Medicine Ueda, Kazuto; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care Suzuki, Toshihiko; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care; Tokyo Women's Medical University Medical Center East, Division of Neonatology Suzuki, Sakiko; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care Miura, Ryosuke; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care Katayama, Akemi; Nagoya University Hospital, Department of Advanced Medicine Ando, Masahiko; Nagoya University Hospital, Department of Advanced Medicine Mizuno, Masaaki; Nagoya University Hospital, Department of Advanced Medicine Hirakawa, Akihiro; Tokyo Medical and Dental University, Department of Clinical Biostatistics Hayakawa, Masahiro ; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care Sato, Yoshiaki; Nagoya University Hospital, Division of Neonatology, Center for Maternal-Neonatal Care
<b>Primary Subject Heading</b>:	Paediatrics
Secondary Subject Heading:	Research methods, Neurology
Keywords:	NEONATOLOGY, Paediatric intensive & critical care < INTENSIVE & CRITICAL CARE, Clinical trials < THERAPEUTICS

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

5  
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17 Suzuki<sup>2</sup>, Ryosuke Miura<sup>2</sup>, Akemi Katayama<sup>1</sup>, Masahiko Ando<sup>1</sup>, Masaaki Mizuno<sup>1</sup>,

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6 18 Akihiro Hirakawa<sup>4</sup>, Masahiro Hayakawa<sup>2</sup>, Yoshiaki Sato<sup>2\*</sup>  
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27 25 <sup>4</sup>Department of Clinical Biostatistics, Graduate School of Medical and Dental  
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30 26 Sciences, Tokyo Medical and Dental University, Tokyo, Japan  
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36 28 **Word count 2,763 words**  
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35 **Abstract**

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

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

51 **Ethics and dissemination:** This study will be conducted in accordance with the

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7 52 Declaration of Helsinki and Good Clinical Practice. The Nagoya University Hospital  
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9 53 Institutional Review Board (No. 312005) approved this study on 13 November 2019.  
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12 54 The results of this study will be published in peer-reviewed journal and reported in  
13  
14  
15 55 international conferences.

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18 56 **Trial registration:** ClinicalTrials.gov: NCT04261335, registered on 7 February 2020,  
19  
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21 57 <https://clinicaltrials.gov/ct2/show/NCT04261335>. Japan Registry of Clinical Trials:  
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24 58 jRCT2043190112, registered on 6 February 2020, [https://jrct.niph.go.jp/latest-](https://jrct.niph.go.jp/latest-detail/jRCT2043190112)  
25  
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27 59 [detail/jRCT2043190112](https://jrct.niph.go.jp/latest-detail/jRCT2043190112)

### 60 61 **Strengths and limitations of this study**

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

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6 **69 Keywords**

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9 70 Hypoxic-ischaemic encephalopathy, neonates, cerebral palsy, hypothermia,  
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12 71 mesenchymal stem cell, Muse cell  
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21 **74 INTRODUCTION**

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24 75 Neonatal hypoxic-ischaemic encephalopathy (HIE) results from acute perinatal  
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27 76 asphyxia and can lead to poor patient outcomes, including death, physical disabilities,  
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30 77 and mental retardation. HIE has an estimated incidence of 1.5 per 1,000 live births  
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33 78 (95% confidence intervals [CI]: 1.3 to 1.7) from the three population-based studies  
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36 79 in United Kingdom, Australia, Sweden carried out since 1980,[1] and the incidence  
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39 80 of moderate or severe HIE has been reported to be 0.37 per 1,000 term live births in  
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42 81 Japan.[2] Birth asphyxia accounts for 23% of global neonatal deaths.[3] Because  
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45 82 HIE is associated with irreversible injury to the central nervous system, its sequelae  
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48 83 such as cerebral palsy, epilepsy, or cognitive impairment could be major persistent  
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51 84 burdens on both patients and their families.

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54 85 The most evidence-based treatment for moderate-to-severe HIE is therapeutic  
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6 86 hypothermia, which maintains a body temperature of 33°C–34°C for 72 hours[4, 5].  
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9 87 However, its effectiveness is limited. A previous study reported that the number  
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12 88 needed to treat was 9 (95% CI: 5–25) for hypothermia therapy to avoid 1 death or  
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15 89 severe disability at 18 months.[6] Therefore, a novel treatment for moderate-to-  
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18 90 severe HIE is warranted.  
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21 91 Regenerative medicine has been developed as a new and effective treatment for  
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24 92 HIE. Preclinical animal studies using umbilical cord blood cells (UCBCs) in neonatal  
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27 93 HIE and stroke rat models have reported effectiveness.[7-9] In addition, some  
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30 94 exploratory clinical studies have shown the safety and feasibility of autologous  
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33 95 UCBCs administration for HIE neonates.[10, 11] However, preparing autologous  
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36 96 UCBCs requires well-equipped facilities and sufficient human resources in birthing  
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39 97 centres, clinics, or hospitals.  
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42 98 From a wide variety of options as candidates for regenerative cells,[12-15] we have  
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45 99 noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells  
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48 100 are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent  
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51 101 markers that self-renew and differentiate from a single cell into each of the three  
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54 102 germ layer cells.[16] They are positive for stage-specific embryonic antigen (SSEA)-  
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6 103 3 and CD105 in the peripheral blood, bone marrow, and organ connective tissues.[17,  
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9 104 18] Muse cells also have a specific immunomodulatory system, represented by  
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12 105 human leukocyte antigen (HLA) -G expression, allowing them to be directly  
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14  
15 106 administered without HLA matching or immunosuppressant agents.[19] Furthermore,  
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18 107 after intravenous administration, Muse cells are distributed to the damaged site by  
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21 108 sphingosine monophosphate (S1P)-sphingosine monophosphate receptor 2  
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24 109 (S1PR2) axis mechanism,[19] and then self-renewed without artificial differentiation  
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26  
27 110 or induction. After migrating, Muse cells differentiate into tissue-compatible cells  
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29  
30 111 according to the microenvironment and remain integrated into the host tissue to  
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32  
33 112 participate in tissue repair.[20, 21] Based on these characteristics, intravenous  
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36 113 administration of allogenic Muse cells is expected to be an effective regenerative  
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39 114 therapy for HIE.  
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42 115 We found that the systemic administration of human Muse cells in the perinatal HIE  
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45 116 rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left  
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48 117 carotid artery, improved learning deficits and motor impairment. In addition, human  
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51 118 Muse cells are localised in the damaged brain and differentiate into neurons.  
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54 119 These effects were much clearer in the Muse cells than in mesenchymal stem cells  
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6 120 (MSCs) without Muse cells subpopulation.[22] Moreover, we confirmed that the  
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9 121 human allogenic Muse cells-based product, CL2020, manufactured by Life Science  
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12 122 Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical  
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15 123 Holdings Corporation, exerted a therapeutic effect with no toxicity in the HIE rat  
16  
17  
18 124 models. To verify the safety and effectiveness of CL2020, LSII has conducted  
19  
20  
21 125 several clinical trials in adult patients with acute myocardial infarction (JapicCTI-  
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23  
24 126 No.: JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103),  
25  
26  
27 127 epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841),  
28  
29  
30 128 amyotrophic lateral sclerosis (jRCT2063200047), and acute respiratory distress  
31  
32  
33 129 syndrome associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-  
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35  
36 130 human clinical trial for acute myocardial infarction was performed in 3 patients and  
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39 131 indicated that CL2020 was safe and significantly improved the left ventricular  
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42 132 ejection fraction.[23] A phase 1/2 open-label trial on adult epidermolysis bullosa  
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45 133 was also recently published. A total of 5 patients received a single injection of  
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48 134 CL2020, and the ulcer size was significantly reduced for up to 3 months.[24]  
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51 135 Nevertheless, the safety and tolerability of Muse cells in neonates are unknown  
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54 136 because they have never been administered to neonates. Based on these results,  
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7 137 we planned the first-in-neonate clinical trial to confirm the safety and tolerability of  
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9 138 CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy.  
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12 139 Hence, we describe the detailed design of an investigator-initiated clinical trial on  
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15 140 neonatal HIE to investigate the safety, tolerability, and efficacy in  
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18 141 neurodevelopmental outcomes at 18 months. This clinical trial is named “The  
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21 142 Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring  
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24 143 cell-based product in Neonatal Hypoxic-Ischemic Encephalopathy Patients with  
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26  
27 144 Therapeutic Hypothermia in the Dose Escalation Clinical Trial” (the SHIELD trial).  
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## 146 **METHODS AND ANALYSIS**

### 147 **Objective and study design**

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

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9 155 **Recruitment and setting**

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12 156 Patient recruitment is done in Nagoya University Hospital or by receiving referrals of  
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15 157 patients from other hospitals in our district. The investigators will obtain written  
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18 158 informed consent from the patients' legal parental authority before screening. After  
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21 159 screening and verifying the patients' eligibility, they will be registered for the trial.  
22

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27 161 **Participants**

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29  
30 162 We will recruit a maximum of 12 neonates with HIE who have received therapeutic  
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32  
33 163 hypothermia. They must meet the following inclusion criteria:

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36 164 1) At least 36 weeks gestational age, and one of the following criteria (i–iii)
- 37  
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39 165 i. Apgar score  $\leq 5$  at 10 minutes
  - 40  
41  
42 166 ii. Continued neonatal resuscitation for at least 10 minutes
  - 43  
44  
45 167 iii. pH  $< 7.0$ , or base deficit  $\geq 16$  mmol/L in any blood sample obtained within  
46  
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48 168 60 min after birth

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51 169 2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]  
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6 170 3) Therapeutic hypothermia initiated within 6 hours after birth and continued for 72

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9 171 hours

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11  
12 172 4) Birth weight  $\geq 1,800$  g

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14  
15 173 5) Heart rate  $\geq 100$ /min, and SpO<sub>2</sub>  $\geq 90\%$  upon enrollment

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18 174 6) Able to provide voluntary informed consent after receiving information about the

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21 175 study (consent will be obtained from a legal proxy).

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27 177 Exclusion criteria are:

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30 178 1) Suspected or confirmed severe congenital abnormalities or chromosomal

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33 179 anomaly

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36 180 2) Planned to undergo surgery or radiation therapy

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38  
39 181 3) Scheduled to take systemic corticosteroids treatment for over 5 days

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41  
42 182 4) Blood glucose  $\geq 200$  mg/dL continuously sustained

43  
44  
45 183 5) Participation in another interventional clinical study

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47  
48 184 6) Suspected or confirmed active and severe infection

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50  
51 185 7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or

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54 186 syphilis serum reaction

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6 187 8) History of severe hypersensitivity or anaphylactic reaction

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9 188 9) Severe complications not related to HIE

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15 190 **Patient and public involvement**

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18 191 Patients' guardians or members of the public were not involved in this study protocol  
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21 192 planning.

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27 194 **Intervention and follow-up**

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30 195 The clinical-grade Muse cell-based product, CL2020 ( $1.5 \times 10^7$  cells/15 mL of frozen  
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33 196 preparation), was produced from human allogenic MSCs by LSII.[26] The CL2020  
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36 197 was produced by exposing MSCs to some stressors, and they were enriched to be  
37  
38  
39 198 positive for both SSEA3 and CD105 but negative for CD45. We will prepare cells  
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42 199 from CL2020 for administration to neonates by centrifuging the product after thawing,  
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44  
45 200 removing the supernatant, and suspending with acetated Ringer's solution. The  
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48 201 patients will receive the prepared cells intravenously once between 5 and 14 days  
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51 202 after birth. We decided to administer as soon as possible within this window (5–14  
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54 203 days after birth) in principle after registration. This study will utilise a 3 + 3 dose-



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6 204 escalation design, setting two cohorts for the injected dose. Subjects in the low-dose  
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9 205 cohort will receive 1.5 million cells, while those in the high-dose cohort will receive  
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11  
12 206 15 million cells. The following treatments will be prohibited during the study:  
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15 207 corticosteroids (prednisolone converted at 2 mg/kg/day or more, and more than 5  
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18 208 days), other human MSC products, processed cell products except for the red blood  
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20  
21 209 cells, other investigational products, and the use of investigational medical devices.  
22  
23  
24 210 Regarding corticosteroid, it affects cell proliferation mediated by RNA transcription  
25  
26  
27 211 [27], we thought that they could affect the function of the administered cells. The  
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29  
30 212 data and safety monitoring board (DSMB) will consist of 3 specialists in paediatric  
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32  
33 213 and perinatal care independent of the trial investigators. The DSMB will be held at  
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35  
36 214 predefined times in both cohorts: at 4 weeks after administering to the first patient  
37  
38  
39 215 and 12 weeks after administering to the third patient in each cohort. The council will  
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42 216 also be held when a product-related severe adverse event occurs or when  
43  
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45 217 investigators consider that it should be convened due to safety concerns. The DSMB  
46  
47  
48 218 will recommend whether this trial should be moved forward or be discontinued.  
49  
50  
51 219 Figure 1 illustrates the framework of this study. The study participants will be  
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54 220 hospitalised for at least 2 weeks after CL2020 administration and followed up for 78  
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221 weeks. The planned visits and data collection are presented in Table 1.

For peer review only

222 **Figure 1**

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											

223 **Study framework**

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

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230

231 **Table 1**232 **Schedule of interventions and assessments**



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4 and platelet count

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6 <sup>c</sup>Blood urea nitrogen, creatinine, lactate dehydrogenase, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, gamma-glutamyl  
7 transpeptidase, total bilirubin, direct bilirubin, creatine kinase, C-reactive protein, sodium, potassium, calcium, phosphorus, and blood glucose level.

8 <sup>d</sup>pH, urine protein, urine occult blood, and urine sugar

9 <sup>e</sup>Bayley Scales of Infant and Toddler Development Third edition

10 <sup>f</sup>Kyoto Scale of Psychological Development 2001

11 <sup>g</sup>Expanded and Revised Gross Motor Function Classification System  
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6 234 **Study endpoints**  
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9 235 The primary outcome is the incidence of adverse events until 12 weeks after  
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11  
12 236 administration. The secondary outcomes are as follows:

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14  
15 237 1) Incidence of composite endpoints (death, continuous respiratory support, or  
16  
17  
18 238 continuous use of vasopressors or pulmonary vasodilators)

19  
20  
21 239 2) Mortality and overall survival

22  
23  
24 240 3) Duration of continuous respiratory support: The definition of respiratory  
25  
26  
27 241 support is the status of conducting artificial ventilation with tracheal intubation.

28  
29  
30 242 4) Duration of continuous use of vasopressors or pulmonary vasodilators:  
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32  
33 243 dopamine, dobutamine, adrenaline, noradrenaline, milrinone, vasopressin,  
34  
35  
36 244 *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide,  
37  
38  
39 245 epoprostenol sodium, nitroglycerin, and alprostadil alfadex

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41  
42 246 5) The Bayley Scales of Infant and Toddler Development Third Edition [28]  
43  
44  
45 247 score at 78 weeks

46  
47  
48 248 6) Developmental quotient as per the Kyoto Scale of Psychological  
49  
50  
51 249 Development 2001 [29] at 78 weeks

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53  
54 250 7) Assessment of postnatal development such as head control, rolling, sitting,  
55  
56  
57 251 crawling, unaided walking, and saying several meaningful words  
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6 252 8) Presence of spasticity: The definition of spasticity is the status of increased  
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9 253 muscle tone or increased deep tendon reflex.  
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11  
12 254 9) Presence of epilepsy: The definition of epilepsy is based on the International  
13  
14  
15 255 League Against Epilepsy.[30]  
16  
17  
18 256 10) Magnetic resonance imaging score: The scoring system is based on the  
19  
20  
21 257 report of Barkovich et al.[31]  
22  
23  
24 258 11) The score of Expanded and Revised Gross Motor Function Classification  
25  
26  
27 259 System [32] at 78 weeks

30 260 In addition, we will collect vital signs and laboratory values for safety assessment  
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32  
33 261 at specific points, as shown in Table 1. In addition, tolerability is determined by  
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35  
36 262 the investigator based on the suggestion of the data safety monitoring board by  
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38  
39 263 confirming a serious adverse event related to the administration of the  
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42 264 investigational product.  
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45 265

### 48 266 **Sample size calculation**

50  
51 267 We did not calculate the sample size with statistical rationale because we used a  
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54 268 3 + 3 dose-escalation design to confirm the safety and tolerability of CL2020. The  
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57 269 scheduled number of enrolled patients is 12.  
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7 2708  
9 271 **Statistical analysis**

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12 272 All analyses are based on an intention-to-treat principle. We will summarise the  
13  
14  
15 273 demographic data using descriptive statistics. The main purpose of this  
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17  
18 274 exploratory clinical trial is “to confirm the safety and tolerability” of the Muse cell  
19  
20  
21 275 product. Therefore, we will analyse adverse events on the safety analysis set  
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23  
24 276 defined as all subjects enrolled in this study and received the investigational cell  
25  
26  
27 277 product. All adverse events will be confirmed for the primary endpoint, and the  
28  
29  
30 278 proportions of the adverse events and their 95% CI based on the Clopper-  
31  
32  
33 279 Pearson method will be calculated. Overall survival, defined as the time from birth  
34  
35  
36 280 to the date of death due to any cause, will be summarised using the Kaplan-Meier  
37  
38  
39 281 method. Descriptive statistics for continuous variables and frequency and  
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41  
42 282 proportion for categorical variables will be calculated for each secondary endpoint.  
43  
44  
45 283 Depending on the endpoint (e.g. the duration of continuous respiratory support,  
46  
47  
48 284 continuous use of vasopressors, or pulmonary vasodilators), it will be  
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50  
51 285 summarised excluding patients who had been using these therapies prior to the  
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54 286 cells administration as necessary. Statistical analysis will be performed using the  
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57 287 SAS software (SAS Institute, version 9.4, North Carolina, USA). Statistical  
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6 288 significance will be defined as  $p < 0.05$ . Some endpoints, including the provision  
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8  
9 289 of respiratory support and the use of vasoactive drugs, may be affected by pre-  
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11  
12 290 enrolment condition, the effects of these potential baseline differences will not be  
13  
14  
15 291 adjusted in the analysis.  
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18 292

### 21 293 **Monitoring and auditing**

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23  
24 294 The monitoring personnel will investigate the progress of this trial and confirm the  
25  
26  
27 295 adequacy of the research procedures. The auditing personnel will check the  
28  
29  
30 296 quality of this trial independent of the investigators, according to the laws,  
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32  
33 297 regulations, study protocol, and standard operating procedures.  
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### 42 300 **Status of this trial**

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45 301 The Ministry of Health, Labour and Welfare accepted this clinical trial notification  
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47  
48 302 as a trial on a new cellular and tissue-based product in January 2020. The first  
49  
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51 303 participant was registered, and CL2020 was administered in March 2020. Three  
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54 304 patients were enrolled into a low-dose cohort, while six were allocated to a high-  
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56  
57 305 dose cohort as of July 2021. Patient recruitment was performed in Nagoya  
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6 306 University Hospital from February 2020 to July 2021, and the study will be  
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9 307 terminated in September 2023.  
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12 308

## 15 309 **ETHICS AND DISSEMINATION**

### 18 310 **Ethical approval**

21 311 This study was approved by the Nagoya University Hospital Institutional Review  
22  
23  
24 312 Board (No. 312005) on 13 November 2019. This study will be conducted in  
25  
26  
27 313 accordance with the Declaration of Helsinki and Good Clinical Practice. The  
28  
29  
30 314 investigators must always obtain approval from the Institutional Review Board  
31  
32  
33 315 about any amendment to the protocol and provide the necessary reasons.  
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### 42 318 **Patient consent for participation**

45 319 The investigators and trained clinical research coordinators will introduce the trial  
46  
47  
48 320 to patients' legal representatives with prepared information sheets and informed  
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50  
51 321 consent forms (Supplementary file). The investigator will obtain written consent  
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53  
54 322 to participate in the trial. Subjects will be identified during the data collection using  
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56  
57 323 a subject identification code. All personnel involved in this study will take the best  
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7 324 possible precautions to ensure the protection of patients' personal information.  
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12 326 **Dissemination**

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15 327 The results of this clinical trial will be published in peer-reviewed journals,

16  
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18 328 presented in conferences, and submitted to clinical trial registries.  
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24 330 **DISCUSSION**

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27 331 This clinical trial aims to evaluate the safety and tolerability of CL2020, a Muse

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29  
30 332 cell-based product, in neonates. When CL2020 was administered intravenously

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33 333 to infant rats, the cells were distributed mainly in the lungs immediately after

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35  
36 334 administration. However, there was no change in respiratory condition or

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38  
39 335 pathological evaluation. Based on non-clinical study data and ongoing clinical

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42 336 trials of CL2020, we decided to implement this clinical trial to ensure safety in

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44  
45 337 neonates.  
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47  
48 338 Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy.

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50  
51 339 Several randomised controlled trials of hypothermia therapy for HIE have been

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54 340 conducted,[33-38] and hypothermia is currently the sole neuroprotective therapy.

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57 341 However, its effectiveness is insufficient, and a novel therapy is required.  
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6 342 Regenerative therapy is the focus of next-generation therapy. Clinical studies  
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9 343 with autologous UCBCs for HIE had been conducted before the development of  
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12 344 CL2020.[10, 11] This UCBCs therapy requires additional equipment and human  
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14  
15 345 resources for its preparation because the newborns' umbilical cord blood has to  
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18 346 be collected at birth, and the patients receive the first dose of prepared UCBCs  
19  
20  
21 347 within 24 hours after birth. In contrast, in our non-clinical study, single intravenous  
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23  
24 348 administration of Muse cells to HIE model rats 3 days after hypoxic-ischaemic  
25  
26  
27 349 injury ameliorated behavioural abnormalities up to 5 months.[22] In a non-clinical  
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30 350 study using CL2020, the treatment effect was exerted at even 7 days after insult  
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33 351 by hypoxic ischaemia. In addition, a single dose of CL2020 administered via the  
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36 352 vein at the subacute (about 9 days after onset) and chronic phases (about 30  
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39 353 days) was effective in a mouse lacunar stroke model.[26] Thus, we set the  
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42 354 administration of Muse cells to human neonates between 5 and 14 days after  
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45 355 birth, which means that physicians and patients' families can afford the time to  
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48 356 decide or prepare the treatment based on the patient's condition or seek other  
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51 357 opinions.  
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54 358 We held a consultation meeting about the main clinical trial design, including the  
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57 359 timing of administration as above with the Japanese regulatory authority,  
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6 360 Pharmaceutical and Medical Devices Agency, and they agreed to our proposed  
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9 361 design for this trial. We will perform a randomised placebo-controlled clinical trial  
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12 362 to evaluate the effectiveness of CL2020 for HIE after confirming the safety and  
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15 363 tolerability of its intravenous administration in neonates.  
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18 364 Herein, we present the overall design of this single-centre, open-label, dose-  
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21 365 escalation clinical trial of Muse cell products in HIE patients with hypothermia.  
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24 366 This clinical trial is the first clinical application of CL2020 in neonates based on  
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27 367 our non-clinical study results. If we can verify that this product is safe and well-  
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30 368 tolerable in neonates, its application may expand to other disorders in neonates  
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33 369 and children.  
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#### 41 42 372 **Funding** 43

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45 373 This work was supported by the Japan Agency for Medical Research  
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48 374 Development [grant number: JP211m0203143].  
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#### 53 54 376 **References** 55 56 57 58 59 60

- 1  
2  
3  
4  
5  
6 377 1 Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal  
7  
8  
9 378 encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*  
10  
11  
12 379 2010;86:329–38.  
13  
14  
15 380 2 Hayakawa M, Ito Y, Saito S, et al. Incidence and prediction of outcome in  
16  
17  
18 381 hypoxic-ischemic encephalopathy in Japan. *Pediatr Int* 2014;56:215–21.  
19  
20  
21 382 3 Lawn JE, Cousens S, Zupan J, et al. 4 million neonatal deaths: When? Where?  
22  
23  
24 383 Why? *Lancet* 2005;365:891–900.  
25  
26  
27 384 4 Shankaran S. Therapeutic hypothermia for neonatal encephalopathy. *Curr*  
28  
29  
30 385 *Treat Options Neurol* 2012;14:608–19.  
31  
32  
33 386 5 Perlman JM, Wyllie J, Kattwinkel J, et al. Part 11: Neonatal resuscitation: 2010  
34  
35  
36 387 international consensus on cardiopulmonary resuscitation and emergency  
37  
38  
39 388 cardiovascular care science with treatment recommendations. *Circulation*  
40  
41  
42 389 2010;122 Suppl 2:S516–38.  
43  
44  
45 390 6 Edwards AD, Brocklehurst P, Gunn AJ, et al. Neurological outcomes at 18  
46  
47  
48 391 months of age after moderate hypothermia for perinatal hypoxic ischaemic  
49  
50  
51 392 encephalopathy: synthesis and meta-analysis of trial data. *BMJ*  
52  
53  
54 393 2010;340:c363.  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4  
5  
6 394 7 Hattori T, Sato Y, Kondo T, et al. Administration of umbilical cord blood cells  
8  
9 395 transiently decreased hypoxic-ischemic brain injury in neonatal rats. *Dev*  
10  
11  
12 396 *Neurosci* 2015;37:95–104.
- 13  
14  
15 397 8 Tsuji M, Taguchi A, Ohshima M, et al. Effects of intravenous administration of  
16  
17  
18 398 umbilical cord blood CD34(+) cells in a mouse model of neonatal stroke.  
19  
20  
21 399 *Neuroscience* 2014;263:148–58.
- 22  
23  
24 400 9 Nakanishi K, Sato Y, Mizutani Y, et al. Rat umbilical cord blood cells attenuate  
25  
26  
27 401 hypoxic-ischemic brain injury in neonatal rats. *Sci Rep* 2017;7:44111.
- 28  
29  
30 402 10 Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord  
31  
32  
33 403 blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr*  
34  
35  
36 404 2014;164:973–9.
- 37  
38  
39 405 11 Tsuji M, Sawada M, Watabe S, et al. Autologous cord blood cell therapy for  
40  
41  
42 406 neonatal hypoxic-ischaemic encephalopathy: a pilot study for feasibility and  
43  
44  
45 407 safety. *Sci Rep* 2020;10:4603.
- 46  
47  
48 408 12 Mikrogeorgiou A, Sato Y, Kondo T, et al. Dedifferentiated fat cells as a novel  
49  
50  
51 409 source for cell therapy to target neonatal hypoxic-ischemic encephalopathy.  
52  
53  
54 410 *Dev Neurosci* 2017;39:273–86.
- 55  
56  
57  
58  
59  
60

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2  
3  
4  
5  
6 411 13 Sato Y, Ueda K, Kondo T, et al. Administration of bone marrow-derived  
7  
8  
9 412 mononuclear cells contributed to the reduction of hypoxic-ischemic brain  
10  
11  
12 413 injury in neonatal rats. *Front Neurol* 2018;9:987.  
13  
14  
15 414 14 Sugiyama Y, Sato Y, Kitase Y, et al. Intravenous administration of bone  
16  
17  
18 415 marrow-derived mesenchymal stem cell, but not adipose tissue-derived stem  
19  
20  
21 416 cell, ameliorated the neonatal hypoxic-ischemic brain injury by changing  
22  
23  
24 417 cerebral inflammatory state in rat. *Front Neurol* 2018;9:757.  
25  
26  
27 418 15 Kitase Y, Sato Y, Ueda K, et al. A novel treatment with stem cells from human  
28  
29  
30 419 exfoliated deciduous teeth for hypoxic-ischemic encephalopathy in neonatal  
31  
32  
33 420 rats. *Stem Cells Dev* 2020;29:63–74.  
34  
35  
36 421 16 Kuroda Y, Kitada M, Wakao S, et al. Unique multipotent cells in adult human  
37  
38  
39 422 mesenchymal cell populations. *Proc Natl Acad Sci U S A* 2010;107:8639–43.  
40  
41  
42 423 17 Dezawa M. Muse cells provide the pluripotency of mesenchymal stem cells:  
43  
44  
45 424 direct contribution of Muse cells to tissue regeneration. *Cell Transplant*  
46  
47  
48 425 2016;25:849–61.  
49  
50  
51 426 18 Wakao S, Akashi H, Kushida Y, et al. Muse cells, newly found non-tumorigenic  
52  
53  
54 427 pluripotent stem cells, reside in human mesenchymal tissues. *Pathol Int*  
55  
56  
57 428 2014;64:1–9.  
58  
59  
60



- 1  
2  
3  
4  
5  
6 429 19 Yamada Y, Wakao S, Kushida Y, et al. S1P–S1PR2 axis mediates homing of  
7  
8  
9 430 Muse cells into damaged heart for long-lasting tissue repair and functional  
10  
11  
12 431 recovery after acute myocardial infarction. *Circ Res* 2018;122:1069–83.  
13  
14  
15 432 20 Wakao S, Kuroda Y, Ogura F, et al. Regenerative effects of mesenchymal  
16  
17  
18 433 stem cells: contribution of Muse cells, a novel pluripotent stem cell type that  
19  
20  
21 434 resides in mesenchymal cells. *Cells* 2012;1:1045–60.  
22  
23  
24 435 21 Dezawa M. Clinical trials of muse cells. In: Dezawa M, eds. Muse cells.  
25  
26  
27 436 Advances in Experimental Medicine and Biology, vol 1103. Tokyo: Springer  
28  
29  
30 437 2018:305–7.  
31  
32  
33 438 22 Suzuki T, Sato Y, Kushida Y, et al. Intravenously delivered multilineage-  
34  
35  
36 439 differentiating stress enduring cells dampen excessive glutamate metabolism  
37  
38  
39 440 and microglial activation in experimental perinatal hypoxic ischemic  
40  
41  
42 441 encephalopathy. *J Cerebral Blood Flow Metab* 2021;41:1707–20.  
43  
44  
45 442 23 Noda T, Nishigaki K, Minatoguchi S. Safety and efficacy of human Muse cell-  
46  
47  
48 443 based product for acute myocardial infarction in a first-in-human trial. *Circ J*  
49  
50  
51 444 2020;84:1189–92.  
52  
53  
54 445 24 Fujita Y, Nohara T, Takashima S, et al. Intravenous allogeneic multilineage-  
55  
56  
57 446 differentiating stress-enduring (Muse) cells in adults with dystrophic  
58  
59  
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- 1  
2  
3  
4  
5  
6 447 epidermolysis bullosa: a phase 1/2 open-label study. *J Eur Acad Dermatology*  
7  
8  
9 448 *Venerol* 2021;35:e528–31.  
10  
11  
12 449 25 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A  
13  
14  
15 450 clinical and electroencephalographic study. *Arch Neurol* 1976;33:696–705.  
16  
17  
18 451 26 Abe T, Aburakawa D, Niizuma K, et al. Intravenously transplanted human  
19  
20  
21 452 multilineage-differentiating stress-enduring cells afford brain repair in a  
22  
23  
24 453 mouse lacunar stroke model. *Stroke* 2020;51:601–11.  
25  
26  
27 454 27 Lester RS. Corticosteroids. *Clin Dermatol*. 1989;7(3):80–97.  
28  
29  
30 455 28 Bayley N. Bayley scales of infant and toddler development. 3rd ed. San  
31  
32  
33 456 Antonio, TX: Psychological Corp 2006:  
34  
35  
36 457 29 Society for the Kyoto Scale of Psychological Development. The Kyoto scale  
37  
38  
39 458 of psychological development 2001: information for standardization and  
40  
41  
42 459 administration. Kyoto, Japan: Kyoto Kokusai Shakai Fukushi Center 2002 [in  
43  
44  
45 460 Japanese].  
46  
47  
48 461 30 Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical  
49  
50  
51 462 clinical definition of epilepsy. *Epilepsia* 2014;55:475–82.  
52  
53  
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56  
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3  
4  
5  
6  
7 463 31 Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome  
8  
9 464 in perinatal asphyxia: evaluation of MR scoring systems. *Am J Neuroradiol*  
10  
11  
12 465 1998;19:143–9.  
13  
14  
15 466 32 Palisano RJ, Rosenbaum P, Bartlett D, et al. Content validity of the expanded  
16  
17  
18 467 and revised Gross Motor Function Classification System. *Dev Med Child*  
19  
20  
21 468 *Neurol* 2008;50:744–50.  
22  
23  
24 469 33 Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat  
25  
26  
27 470 perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361:1349–58.  
28  
29  
30 471 34 Jacobs SE, Morley CJ, Inder TE, et al. Whole-body hypothermia for term and  
31  
32  
33 472 near-term newborns with hypoxic-ischemic encephalopathy. *Arch Pediatr*  
34  
35  
36 473 *Adolesc Med* 2011;165:692–700.  
37  
38  
39 474 35 Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild  
40  
41  
42 475 systemic hypothermia after neonatal encephalopathy: multicentre  
43  
44  
45 476 randomised trial. *Lancet* 2005;365:663–70.  
46  
47  
48 477 36 Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for  
49  
50  
51 478 neonates with hypoxic–ischemic encephalopathy. *N Engl J Med*  
52  
53  
54 479 2005;353:1574–84.  
55  
56  
57  
58  
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60

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2  
3  
4  
5  
6 480 37 Simbruner G, Mittal RA, Rohlmann F, et al. Systemic hypothermia after  
7  
8  
9 481 neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics*  
10  
11  
12 482 2010;126:e771–8.

13  
14  
15 483 38 Zhou W, Cheng G, Shao X, et al. Selective head cooling with mild systemic  
16  
17  
18 484 hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter  
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21 485 randomized controlled trial in China. *J Pediatr* 2010;157:367–72.

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#### 25 26 27 487 **Contributorship Statement**

28  
29  
30 488 YS is the principal investigator in this trial and has access to all data. NM, SSh,  
31  
32  
33 489 KU, TS, AK, MA, AH, and YS developed the study protocol. SSh and YS  
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36 490 participated in the conception and design of the study. AK is a quality control  
37  
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39 491 monitor, MA is responsible for data management, and AH supervises the  
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42 492 statistical analysis. NM, SSh, and MM supported the preparation and  
43  
44  
45 493 management of this study. KU, TS, SSu, RM, MH, and YS helped recruit and  
46  
47  
48 494 evaluate patients and prepare cells for administration. NM drafted and revised  
49  
50  
51 495 the manuscript. SSh and YS have revised the manuscript. All authors read and  
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54 496 approved the final manuscript.

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6 498 **Competing Interests**  
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8  
9 499 SSh, MM, and YS have collaborative projects with research funding from LSII for  
10  
11  
12 500 perinatal diseases. SSh and AH receive fees based on a consultation contract  
13  
14  
15 501 from LSII. SSh, TS, MM, MH, and YS have a patent for the application of Muse  
16  
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18 502 cells in the treatment of HIE and other indications. LSII provided CL2020 for this  
19  
20  
21 503 clinical trial free of charge.  
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30 506 **Acknowledgements**  
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32  
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34  
35  
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37  
38  
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40  
41  
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43  
44  
45 511 study. We would like to thank Editage ([www.editage.com](http://www.editage.com)) for English language  
46  
47  
48 512 editing.  
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54 514 **Data availability statement**  
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57 515 The datasets generated and analysed during this study will not be publicly  
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6 516 available due to the confidentiality clause. This clinical trial is the first study for  
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9 517 neonates in Japan to investigate the safety and dosage of Muse cell-product in a  
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12 518 small group for ischemic hypoxic encephalopathy conducted in single centre.  
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15 519 Data on individual subjects obtained in this clinical trial will not be disclosed at  
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18 520 this time for the protection of personal information, because the risk of “re-  
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21 521 identification” is high due to small number of enrolled patients.  
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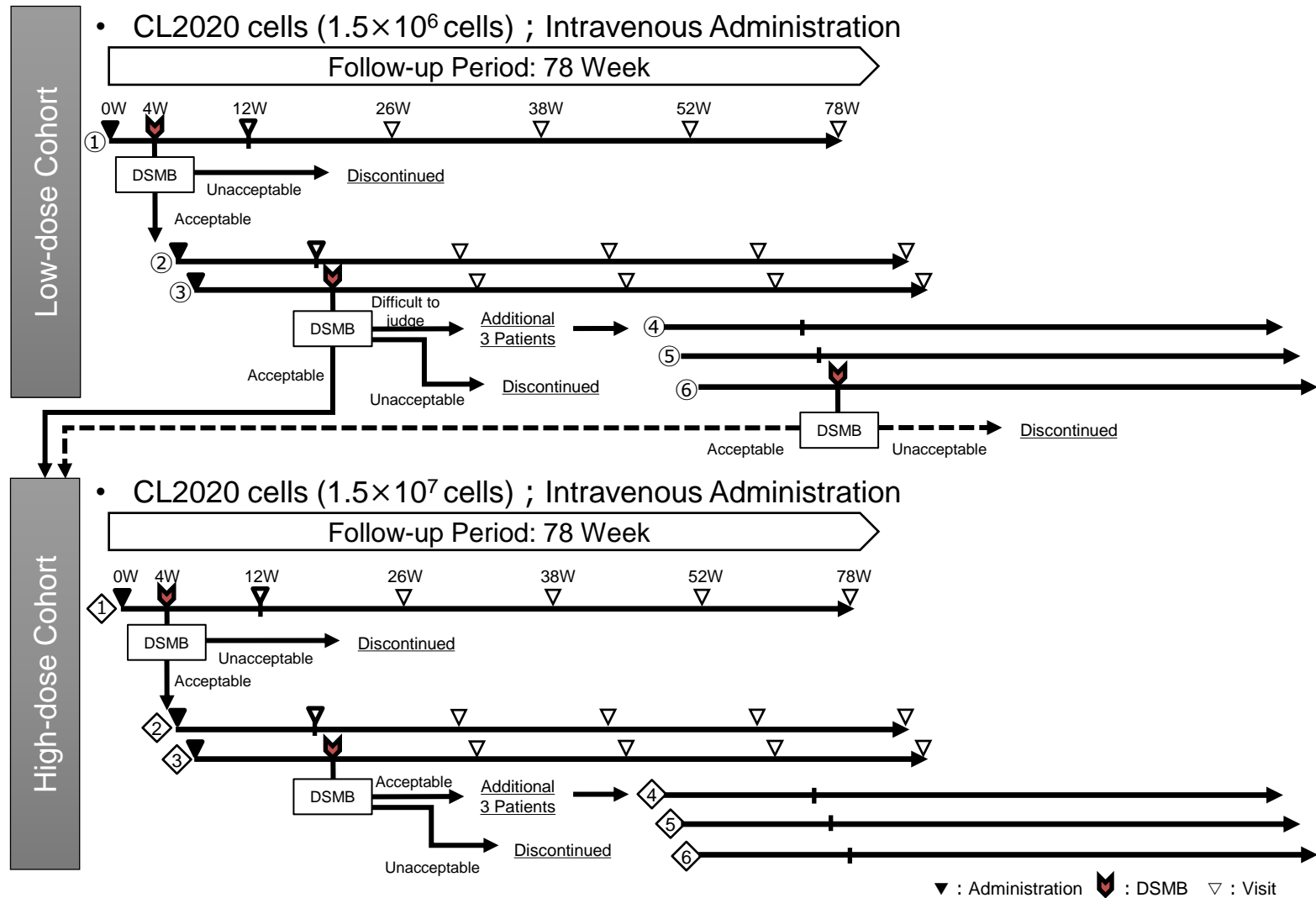


Figure 1 Study framework

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医師主導治験

## 説明文書・同意文書

### ～「低体温療法を実施した新生児低酸素性虚血性脳症に対する CL2020の安全性及び<sup>にんようせい</sup>忍容性を検討する用量<sup>ぜんそう</sup>漸増臨床試験」～

現在、私たち（担当医師）は、患者さんの協力を得て、開発中の<sup>さいせいりょう</sup>再生医療  
<sup>とうせいひん</sup>等製品であるCL2020の安全性と効き目を調べるための臨床試験（<sup>ちけん</sup>治験  
）に取り組んでいます。

今回、あなたにこの治験の内容について説明させていただきます。この説  
明文書は、私たちの説明をおぎない、あなたの理解を深めるためのもの  
なのでよくお読みになり、治験にご協力いただけるかどうかご検討くだ  
さい。

この治験に参加するかどうかはあなたの自由です。治験に参加した後  
でも、いつでも自由にやめることができます。もし参加されなくても、  
あなたやあなたのお子さんが不利益を被ることは全くありません。

この治験に参加するかどうかを決めていただくためには、あなたに治験  
の内容についてできるだけ多く知っていただくことが必要です。説明の中  
でわかりにくい言葉や疑問、質問がありましたらどんなことでも遠慮なくお尋  
ねください。



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## 1. 治験について

新しい薬や製品（再生医療等製品<sup>注</sup>）：組織・細胞加工製品または遺伝子治療製品<sup>せいひん</sup>）が患者さんの治療に使われるようになるまでには、薬や製品としての治療効果と安全性を十分に調べなければなりません。新しい製品の場合は、まず様々な細胞の中から「製品の候補」を選び出し、いろいろな動物や病気のモデルを使い、「製品の候補」の性能、治療効果と安全性について調べます。そして期待される結果が得られた場合は、「製品の候補」が人の病気に役立つかどうかを患者さんに使っていただいて調べます。

<sup>注</sup> 再生医療等製品：以下に当てはまる製品のことで、政令<sup>せいれい</sup>（内閣が決めるルール）で定められたものをいいます。

- ①人または動物の細胞に培養などの加工をした製品（体の表面に付けたり体内に入れたりして、体の形やはたらきを元の状態に近づけるもの、および病気の治療・予防を目的として使用するもの）
- ②遺伝子治療を目的として、体内に入れる（細胞内へ導入する）ことで使用する製品（遺伝子治療用製品）

このように、人での性能、治療効果と安全性を調べる試験のことを「臨床試験」と言い、その中でも国（厚生労働省）に「製品」として認めてもらうために行う臨床試験を「治験」と呼んでいます。また、治験には、製薬会社や医療機器メーカーなどの企業が主体となって実施する治験と、医師自らが計画を立てて実施する治験（医師<sup>いし</sup>主導<sup>しゅどう</sup>治験<sup>ちけん</sup>）があり、今回あなたに説明する治験は、この「医師主導治験」です。治験には一般の治療と異なり、研究的な側面があります。また、治験で使われる製品を「治験製品<sup>ちけんせいひん</sup>」といいます。

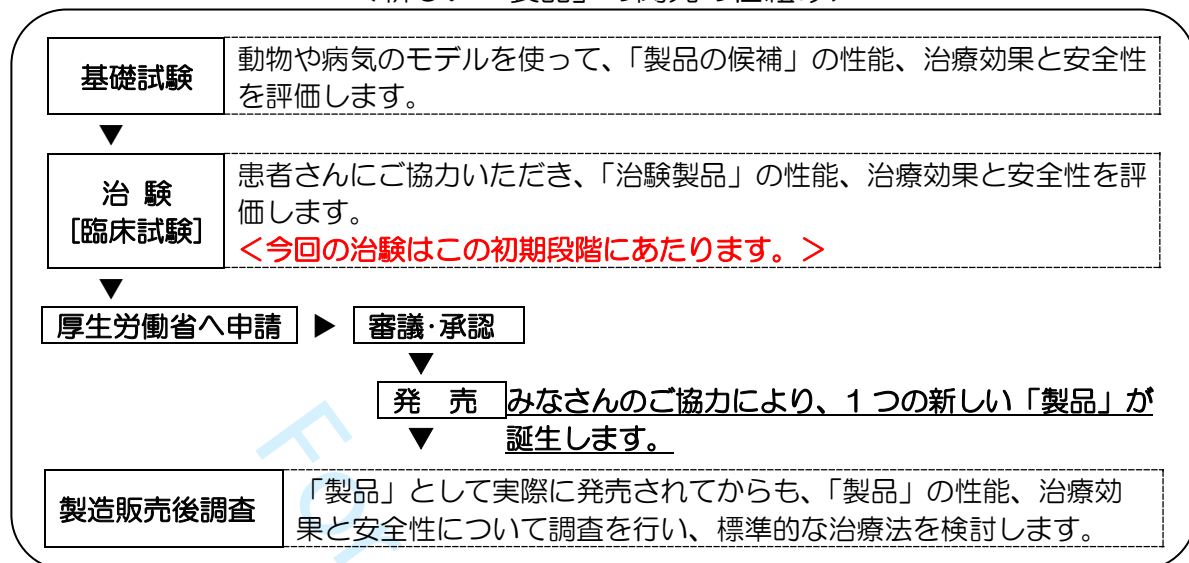
このような治験によって得られた結果は、患者さんのプライバシーに関わる情報を切り離した上で報告書にまとめ、最終的には厚生労働省などの規制当局に提出する資料となります。またこれらの資料は、学術論文の発表、「製品」として販売される際の添付文書<sup>注</sup>といった形で社会に還元され、将来同じ病気になった多くの患者さんの治療に役立つこととなります。また、現在私たちが使用しているくすりを含めた「製品」は、すべて長い年月をかけて、このような治験を積み重ねることによって生み出されたものです。

<sup>注</sup> 再生医療等製品を販売する際に添付することが義務付けられている文書で、法令によって定められた項目（再生医療等製品の場合は、「効能、効果または性能」「用法および用量または使用方法」「不具合・副作用」「臨床試験成績」など）が書かれています。

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For peer review only

## &lt;新しい「製品」の開発の仕組み&gt;



治験は、国が定めた「再生医療等製品の臨床試験の実施の基準に関する省令（再生医療等製品 GCP 省令）」というルールに従い、患者さんの権利が守られ、不必要な危険にさらされないよう倫理的に、かつ科学的妥当性をもって行う必要があります。

また、この治験を行うことについては、病院内に設置されている治験審査委員会ちけんしんさいいんかいで審査を受け、すでに承認されています。

## 「名古屋大学医学部附属病院 治験審査委員会」について

設置者：名古屋大学医学部附属病院長

住 所：〒466-8560 愛知県名古屋市昭和区鶴舞町 65

患者さんの安全を守る立場から、治験の内容が科学的および倫理的に妥当であるかどうかの審議を行い、病院長に意見を述べる委員会です。今後、この治験が行われている間に新しい情報がわかった場合には、その内容を確認して、引き続きこの治験を正しく安全に行うことができるかどうかを審査していきます。

なお、治験審査委員会の手順書（審査の進め方を示した文書）や議事概要（話し合いの記録）、委員の名簿などは、当院の先端医療・臨床研究支援センター（中央診療棟地下1階）に直接お越しいただければ、いつでも内容を確認していただくことができます。また、各文書の内容は先端医療・臨床研究支援センターのホームページでも公開しております。（URL：<http://www.nu-camcr.org/cms/ctc/public/giji/>）

## <医師主導治験とは>

通常の治験は、製薬会社などの企業が医療機関に治験を依頼して行われます。しかし、今回の治験は、私たち医師が自ら治験を実施するもので「医師主導治験」と呼びます。医師主導治験は、通常、製薬会社が行う治験の業務を医師自らが行うもので、治験中に発生する検査費用の負担方法が異なるなど、製薬会社が行う治験とは少し異なる点があります。

具体的には、製薬会社を実施する場合は製薬会社が検査費用の全額を負担しますが、医師主導治験の場合は患者さんの健康保険を適用します。費用負担に関する詳細は「11. 費用の負担について」を参照してください。

## 2. あなたのお子さんの病気（症状）について

ていさんそせいきよけつせいのうしょう  
低酸素性虚血性脳症とは、出産直前や出産の際に、何らかの原因で胎盤血流に問題が生じ、赤ちゃんの脳に十分な血液や酸素が届かなくなり、脳に傷害を受けてしまう疾患です。脳に傷害を受けることで、今後、うんどうまひ運動麻痺、てんかん（脳の細胞が通常とは異なる活動をすることによって引き起こされる症状）、せいしんはったつちたい精神発達遅滞などの神経症状が起こる可能性があります。中等症～重症の場合は、死亡や重度の後遺障害をきたしてしまう可能性も高いことが知られています。

## 3. 現在の治療法について

中等症～重症の新生児低酸素性虚血性脳症に対する治療としては、生後6時間以内に開始して72時間継続するていたいおんりょうほう低体温療法があり、あなたのお子さんに対してもすでにこの治療が行われています。低体温療法は、体温を一定温度下げることによって脳の負担を減らし、脳を保護する治療法です。この治療法は、新生児低酸素性虚血性脳症に対する有効性が確認されており、日本をはじめとする多くの国で治療法として取り入れられていますが、その効果は限定的であり、その他の有用な治療法もありません。そのため、低酸素性虚血性脳症に対する新規治療法の開発が望まれています。

## 4. 「CL2020」について

株式会社生命科学インスティテュートが開発中の「CL2020」は、ヒト（同種 ██████████  
██████████ SSEA-3陽性間葉系幹細胞<sup>ようせいかんようけいかんさいぼう</sup>を多く含む細胞製剤です。ただし、今回の治験では、CL2020の細胞以外の成分を除去後、酢酸リンゲル液で再度分散することとして  
ています。

「SSEA-3」とは細胞の表面に存在する物質の一つであり、発生（受精卵が細胞分裂を繰り返し、次第に各器官が形成され個体ができ上がっていく過程）のごく初期の過程にある細胞だけに存在すると考えられていました。ところが2010年に、成人の体内の細胞から SSEA-3 を持つ細胞（SSEA-3 陽性細胞）が発見され、この細胞は、その特性から「Muse細胞<sup>ミューズさいぼう</sup>」と命名されました。その後の研究で、Muse細胞は、さまざまな細胞になる能力（多能性）を持っているものの、腫瘍化<sup>しゅようか</sup>（がんに変化すること）しないこと、体内の各所にごく少数存在し、臓器や組織が傷害を受けると傷害部位に多く集まることなどが明らかにされており、私たちの体にもともと備えられている自然の修復機能を担う細胞ではないかと考えられています。

CL2020は、ヒトの ██████████ 細胞（ヒト ██████████ 細胞）から SSEA-3 陽性細胞を培養して製造した細胞製剤です。 ██████████

██████████ ██████████ 心筋梗塞<sup>しんきんこうそく</sup>（検証的試験  
※2）、<sup>せきすいそんしょう</sup>脊髄損傷、筋萎縮性側索硬化症（ALS）、及び COVID-19 に伴う急性呼吸  
<sup>きゅうはく</sup>窮迫症候群（様々な疾患が原因となり、重度の呼吸不全となる症状の総称：ARDS）  
の患者さんを対象とした治験を実施中であり、また、心筋梗塞<sup>しんきんこうそく</sup>（探索的試験※3）、脳梗塞、及び<sup>ひょうひすいぼうしょう</sup>表皮水疱症（日常生活における軽微な外力によって皮膚のただれ（びらん）  
や水ぶくれ（水疱）を生じる遺伝性の皮膚病）の患者さんを対象とした治験が完了しています。また、同じ時点で、本治験では9例の新生児に投与されました。

これまでの動物を用いた検討で、生後間もないラットを用いた低酸素性虚血性脳傷害モデルに CL2020 の細胞を投与したところ、成長後の行動学的異常が軽減しました。また、生後間もないラットを用いた低酸素性虚血性脳傷害モデルでの脳室拡大（傷害を受けた脳が小さくなり、空洞になる状態）や神経萎縮を軽減する傾向も確認できました。しかし、現時点ではヒトの低酸素性虚血性脳症に対する有効性は明らかになっていません。

※1 プラセボ（偽薬）：見た目は薬や製品と同じで、薬や製品としての効き目のある成分を全く含

んでいないもの。本来、体に対する影響はないはずですが、人によっては何らかの効き目や症状がみられることがあります。

※2 検証的試験：多くの患者さんにご協力いただき、主にプラセボ（偽薬）や既存の治療方法と比較して治験製品の効き目と安全性を評価する試験です。

※3 探索的試験：少数の患者さんにご協力いただき、治験製品の効き目と安全性を評価し、治験製品の適切な量や使い方を決める試験です。

## 5. 治験の目的について

この治験の目的は、低体温療法を実施した新生児低酸素性虚血性脳症の患者さんにご協力いただき、CL2020の細胞の安全性を確認することです。

この治験は、当院のみで実施し、最大12人の患者さんに参加していただく予定です。

## 6. 治験の方法について

この治験では、患者さんの安全を確保しつつ、科学的に適切な評価を行うために、以下のような基準を設けています。

- 治験に参加していただける方の主な基準
  - 1) 新生児低酸素性虚血性脳症と診断された患児
  - 2) 在胎36週以上で出生した患児
  - 3) 生後6時間以内に低体温療法を開始し、72時間程度の冷却を実施した患児
  - 4) 出生体重が1,800g以上の患児
  - 5) 生後14日以内に治験に登録できる患児
  - 6) 同意取得時の代諾者である親権者が成人（未成年であっても婚姻している場合も含む）である患児
- 治験に参加していただけない方の主な基準
  - 1) 先天的な異常や染色体異常が認められている又は疑われる患児
  - 2) 生後1ヵ月以内に切開を伴う手術（気管切開や胃瘻形成は除く）や放射線療法が予定されている患児
  - 3) 生後1ヵ月以内に全身作用を目的とする副腎皮質ステロイドによる治療を連続5日以上継続することが予定されている患児

- 4) 血糖値が継続して高い患児
- 5) 他の臨床試験に参加している患児
- 6) 活動性の感染症を有している又は疑われる患児
- 7) HBs抗原、HCV抗体、HIV抗体、HTLV-1抗体又は梅毒血清反応のいずれかが陽性の患児
- 8) 重い過敏症又はアナフィラキシー反応の経験のある患児
- 9) 新生児低酸素性虚血性脳症と関連しない、心臓、肝臓、腎臓、肺、血液などに重い病気のある患児
- 10) その他、医師が不相当と判断した場合

また、ここに挙げた主な基準の他に、治験参加の同意をいただいてから検査を行い、確認する必要がある基準がいくつかあります。そのため、同意をいただいても、その検査結果によっては治験に参加できない場合があります。なお、治験が始まってからも、基準を満たしていないことがわかった場合や、治験を続けられない方がよいと私たちが判断した場合は、途中で治験を中止することもありますので、ご了承ください。



## 治験製品の投与方法と投与量について

この治験では、治験製品であるCL2020の細胞を院内で調製し、1回静脈内投与します。その細胞の投与量は、2つの群に分かれており、治験に参加された順番に従って、以下のどちらかの用量を投与します。低用量群は、体重換算すると、これまでの成人での治験で投与されてきた投与量と同量になり、高用量群は、成人に投与した投与量よりも体重換算で10倍の投与量になります。なお、私たちがCL2020を本治験のために調製するにあたっては、CL2020の細胞以外の成分を除去後、酢酸リンゲル液で再度分散することとしています。

### ■低用量群 150万個の細胞：3～6名

この治験に参加された3人目までの方は、「CL2020」1バッグ（約1,500万個の細胞）分の細胞を酢酸リンゲル液で希釈し、そのうちの（約150万個の細胞）を静脈内投与します。患者さんの安全性を確認しながら、6人目の方までこの用量での投与を行う場合があります。

### ■高用量群 1,500万個の細胞：3～6名

低用量群での安全性確認後に最大6名の患者さんに「CL2020」1バッグ（約1,500万個の細胞）分の細胞を酢酸リンゲル液で希釈し、その（約1,500万個の細胞）を静脈内投与します。

## 治験のスケジュール

この治験への参加に同意された後、治験に参加いただけるかどうかを確認するための診察や検査を行います。

その結果、参加の基準を満たした患者さんに治験製品の投与を日齢5から14のどこかで1回行います。

治験製品投与後は78週（1年半）後まで、次ページのスケジュール表にしたがって観察や検査などを行います。そのため、治験の参加予定期間は1年半となります。その後は治験全体が終了するまで、あなたのお子さん体の状態を診察または電話などで確認させていただきます。



## ★1 臨床検査（血液検査・尿検査）

1 回の採血量は約2~3mLです。

血液	<b>血液学的検査</b> 赤血球数、ヘモグロビン、ヘマトクリット、白血球数、白血球分画、血小板数 <b>血液生化学的検査</b> AST、ALT、ALP、 $\gamma$ -GTP、総ビリルビン、直接ビリルビン、LDH、BUN、クレアチニン、クレアチニンキナーゼ、Na、K、Cl、P、CRP、血糖値 <b>血液ガス</b> pH、二酸化炭素分圧（ $pCO_2$ ）、過剰塩基（BE） <b>凝固検査</b> PT、APTT、PT-INR、フィブリノーゲン、アンチトロンビンⅢ <b>感染症検査</b> HBs抗原、HCV抗体、HIV抗体、HTLV-1抗体、梅毒血清反応
	随時尿 タンパク、潜血、糖、pH

★2 痙性（筋緊張や深部腱反射の亢進などの状態）を確認します。

★3 定頸（両脇の下に手を入れて体を支えたときに頭がまっすぐに支えられていること）、寝返り、坐位（支えなしで、手をつかないで背を伸ばして座れること）、はいはいの状態について確認します。

★4 独歩（ものにつかまらず、一人で歩けること）、有意語（意味のある単語を一つ以上言うこと）について確認します。

★5 対面式乳児発達検査：臨床心理士により、発達診断検査を行います。2つの発達診断検査を行います。それぞれの検査で1~2時間程度必要となるため、2日に分けて実施する場合があります。

★6 頭部MRI：磁気共鳴画像検査。エックス線は使用せず、強い磁石と電磁波を使って体内の状態を断面像として描写する検査です。

★7 GMFCS-E&R：粗大運動能力分類システム。子供の坐位や移動を中心とした粗大運動能力をもとに、機能レベルを5段階に分類化し評価します。

また、治験に参加する前に通常の診療で実施した検査結果がある場合は、治験のための検査をあらためて行わずに、その検査結果を使用させていただくことがあります。

なお、あなたのお子さんの体の状態によっては、治験で必要とされる検査以外に、私たちの判断で検査を追加する場合がありますのでご了承ください。

## 7. 予測される心身の健康に対する利益と不利益について

### 予測される利益について

新生児低酸素性虚血性脳症に対し、CL2020の細胞を投与することの有効性は現時点で明らかになっていません。このため、この治験に参加することであなたのお子



症状を示す可能性があります。

• **異所性組織形成・造腫瘍性**

本治験製品の構成成分である間葉系幹細胞は、様々な組織（骨、軟骨、脂肪細胞等）への分化能を有することから、異所性組織形成（目的としない組織になってしまうこと）や腫瘍（がん等）があらわれる可能性が否定できません。ただし、これまでに実施した動物を用いた検討で異所性組織形成や腫瘍の形成は認められませんでした。

• **免疫抑制作用による感染症の増悪**

本治験製品には、免疫抑制作用（ヒトに備わっている、細菌やウイルスなどに抵抗する力を弱める作用）があるため、細菌やウイルスによる感染にかかりやすくなる可能性や、感染にかかった場合に症状が悪化する可能性が否定できません。

• **類似する製品で認められたリスク**

本治験製品に類似した細胞を含む製品を投与した際のリスクとして、細胞塞栓（投与した細胞のかたまりが血管に詰まった状態）、血栓形成（血管の中に血のかたまりが生じた状態）、血管内溶血（血管の中で、血球が壊れた状態）、並びに免疫応答に起因する事象が報告されています。具体的には、本治験製品に類似した細胞を含む製品を用いて日本国内で実施された臨床試験で下記のような有害事象が認められています。

① **肺の循環障害による有害事象**

呼吸困難（2.6%）、低酸素症（2.6%）、酸素飽和度低下（2.6%）、失神寸前の状態（2.6%）、痰貯留（2.6%）

② **血管内溶血による有害事象**

貧血（10.3%）、血中乳酸脱水素酵素（LDH）増加（10.3%）、γ-グルタミルトランスフェラーゼ（γ-GTP）増加（7.7%）、血中ビリルビン（T-Bil）増加（5.1%）、ヘモグロビン（Hb）減少（5.1%）、トランスアミナーゼ（ALTまたはAST）上昇（2.6%）、ハプトグロビン減少（2.6%）

③ **細胞塞栓および局所循環障害**

血栓性微小血管症（細い血管内に血小板のかたまりが生じ、血小板が破壊されて



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• 動物に対する試験の結果

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以上から、今回の治験で重大な副作用が発現する可能性は低いと考えられます。しかし、現時点では予測できない症状が発生する可能性もあります。また、この治験への参加に伴い、通常の診療に比べて、お子さんのからだの状態や神経発達を確認するための検査や、通院の回数が増える負担がかかることが予想されます。治験期間中はもちろん、治験を終了（中止）した後も、何か気になる症状を感じたときは、その症状の程度にかかわらず、すぐに私たちにお知らせください。あなたのお子さんの体の状態を確認して、検査や治療が必要かどうかを判断し、適切に処置いたします。

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ここまでの説明を読んで、あなたがこの治験ではなく通常の治療（経過観察、並びに神経症状が出現した場合はその対症療法）を希望される場合、また、治験に参加された後に中止となった場合は、あなたのお子さんにとって、最も良いと思われる治療方法を、あなたと相談の上で決めていきます。



## 8. 治験の中止について

あなたに治験参加の同意をいただいた後でも、次のような場合には治験へ参加していただけないことや、治験を中止することがありますのでご了承ください。

- 1) あなたが治験の中止を申し出た場合
- 2) 検査などの結果、あなたのお子さんの症状や体の状態が治験への参加基準に合わないことがわかった場合
- 3) 参加いただいている途中で、あなたのお子さんの体の状態の変化やその他の理由により治験をやめたほうがよいと私たちが判断した場合
- 4) あなたのお子さんが予定通りに来院できなくなった場合
- 5) 厚生労働省や私たちの判断によりこの治験が中止される場合

## 9. この治験に関する新たな情報が得られた場合について

治験に参加されている期間中、あなたのお子さんの健康や治験継続の意思に影響を与えるような新たな情報が得られた場合は、すみやかにお知らせいたします。その場合には、治験を続けることに関してもう一度参加の意思を確認させていただくことがあります。

## 10. 副作用などの健康被害が生じた場合の補償について

この治験は、これまでの結果に基づいて科学的に計画され、慎重に行われます。もしもこの治験に参加している間に、あなたのお子さんに副作用などの健康被害が生じた場合には、すぐにお知らせください。ただちに最善と考えられる治療や処置を行い、適切に対処します。その際の医療費は、通常診療と同様に、あなたのお子さんが加入している健康保険が適用されます。医療費・医療手当（治療に伴う医療費以外の費用を補てんする一定の金額）が支払い可能な場合もありますので、適宜ご確認ください。

また、この治験に参加することによって起こった重度の後遺障害（医薬品副作用被害救済制度の後遺障害1級、2級に相当する）および死亡に至るような健康被害に対しては、この治験で用意している補償制度により補償金の支払いが受けられることがあります。その際あなたが、健康被害と治験との関連性を証明する必要はありません。

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4 補償を受けることができるのは、治験への参加に同意いただいた（同意文書に署名  
5  
6 した）日以降に生じた、あなたのお子さんが治験に参加したことによる健康被害に限  
7  
8 られます。

9  
10 ただし、下記の場合などは、補償されないことがあります。

- 11 1) 健康被害と治験との法律的な因果関係が存在しない場合
- 12 2) 治験製品の効き目が不十分であることによって症状が悪化した場合
- 13 3) その健康被害があなたの故意または重大な過失によって生じた場合

14  
15  
16  
17 また、健康被害と治験との関連性がなく、治験とは別に賠償責任が生じている場合  
18  
19 は、補償の対象とはなりません。その場合、損害賠償請求のための訴えを起こすこと  
20  
21 も可能です。補償の詳細につきましては、「治験に係る補償制度の概要について」を  
22  
23 ご覧ください。

24  
25 健康被害が生じた場合は、病院の相談窓口（21 ページ参照）までご連絡ください。

## 26 27 28 29 11. 費用の負担について

30  
31  
32 この治験で使用するCL2020は、提供会社（株式会社生命科学インスティテュー  
33  
34 ト）から無償で提供されます。その他の医療費（初診料や再診料、入院費用、薬剤費  
35  
36 用、検査代など）については、あなたのお子さんが加入する健康保険が適用されます。

37  
38 また、治験参加に伴う交通費などの負担を軽減するため、この治験で規定された来  
39  
40 院1回（入院1回）につき負担軽減費として7,000円を、あなたの希望を確認した  
41  
42 上でお支払いします。支払いは、原則として来院された月ごとにまとめて、その翌々  
43  
44 月までに、あなたの指定する銀行、信用金庫または農業協同組合の口座に東海国立大  
45  
46 学機構から振り込むこととなります。なお、口座番号、電話番号などの個人情報  
47  
48 は厳重に管理し、振り込みの手続きのみに適切に使用いたします。

49  
50 なお、この負担軽減費の受け取りについては、税法上、雑所得としての取扱いを受  
51  
52 けることとなります。年間の受け取り額により確定申告を行う必要がありますので、  
53  
54 あらかじめご了承ください。また、生活保護の支給を受けられている方は、支給額が  
55  
56 変更される場合がありますので、ご注意ください。詳しくは管轄の福祉事務所にご相  
57  
58 談ください。

## 12. プライバシーの保護について

この治験で得られた結果は、私たちが報告書にまとめて、厚生労働省などの規制当局に提出する資料となります。また、治験の結果は学会や医学雑誌などに発表されることや治験製品の提供会社に提供することもあります。さらに、臨床試験情報のデータベースである臨床研究実施計画・研究概要公開システム (<https://jrct.niph.go.jp/>)などで公表されます。ただし、いずれの場合にも、あなたやあなたのお子さんの個人情報（名前や住所、電話番号など）が公表されることは一切ありません。また、治験により得られたデータが他の目的に使用されることも原則ありません（使用する場合は、別途同意を取得させていただきます）。例えば、この治験のためにあなたのお子さんから提供された血液や尿などの検体は他の目的で使用することはなく、検査を終えた後に廃棄いたします。

また、この治験が適正に行われているかどうかを確認するために、治験の関係者（当院の担当者、開発業務受託機関の担当者、厚生労働省などの規制当局の職員、当院の治験審査委員会の委員、治験製品の提供会社の担当者など）が、あなたのお子さんの診療に関する記録（他の診療科の分や治験参加以前の期間も含む）を閲覧することになります。しかし、このような場合でも、これらの関係者には守秘義務が課せられていますので、あなたやあなたのお子さんのプライバシーにかかわる情報は保護されます。

あなたのお子さんが他院や他の診療科に受診されているもしくは受診される場合、あなたのお子さんの安全を守るため、また、治験による影響の有無を確認するために、治験に参加していることを担当医に連絡し、治療の内容（使用した薬など）について問い合わせをさせていただくことがあります。

なお、最後のページにあります同意文書に署名されますと、上記の治験の関係者による閲覧、および私たちが必要と判断したあなたの診療情報（治療内容など）を入手することについてご了解いただいたこととなります。

## 13. 治験への参加の自由と同意撤回の自由について

この治験に参加するかどうかについては、ご家族と相談するなどして十分に考えていただき、あなた自身の自由な意思でお決めください。また、一度同意していただい

1  
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4 今後も、いつでも自由に同意を撤回して治験への参加をやめることができますので、  
5  
6 遠慮なく私たちに伝えてください。この治験に参加されなくても、あなたやあなた  
7  
8 お子さんが不利益を被ることは一切ありません。これまで通り、あなたのお子さんに  
9  
10 にとって最も良いと思われる治療法を、あなたと相談の上で決めていきます。

11  
12 ただし、治験製品を使用された後に治験への参加をやめられる場合は、あなたのお  
13  
14 子さんに対する治験製品の影響を確認し、あなたのお子さんの健康管理のために、必  
15  
16 要に応じて適切な検査を受けていただき、医学的に問題がないかを確認させていただ  
17  
18 く場合があります。

19  
20 また、途中で治験への参加の同意を撤回し、中止した場合は、それまでに得られた  
21  
22 記録や結果の使用の可否について、あなたの意思を確認させていただきます。

## 23 24 25 14. 守っていただきたいことについて

26  
27 この治験に参加していただける場合は、次のことをお守りください。

- 28  
29
- 30 ① 治験に参加している間は、私たちの指示通り、スケジュールに従い、必ず診察、  
31  
32 検査、投薬などを受けてください。もし、来院予定日に来院できない場合は、必  
33  
34 ず私たちに連絡してください。
  - 35  
36 ② 他の薬との組み合わせで治験製品の作用が強まったり弱まったりすることがあ  
37  
38 りますので、普段服用している薬や、他の病院からもらっている薬がある場合に  
39  
40 は、治験に参加される前に必ず私たちに伝えてください。  
41  
42 また、治験中に他の病院で治療を受ける場合や新たに薬を使用される場合は、事  
43  
44 前に私たちに相談してください。緊急の場合は、同意をいただいた後にお渡しす  
45  
46 る「治験参加カード」を必ず医師または看護師、薬剤師にお見せください。
  - 47  
48 ③ 治験期間中だけでなく、治験終了後も有害事象が生じたり、何か心配事があった  
49  
50 場合には、いつでも連絡してください。
- 51  
52

## 53 54 15. 利益相反について

55  
56 り えき そう はん シーオーアイ  
利益相反（COI：Conflict of Interest）とは「主に経済的な利益関係によって  
57  
58 公正かつ適正な判断が ゆが 歪められてしまうこと、または、歪められているのではない  
59  
60 かと疑われかねない事態」のことを指します。具体的には、製薬企業や医療機器メー

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カーから研究者（治験責任（分担）医師等）へ提供される謝金、研究費、サービス及び物品等、ならびに研究に関連する企業の株式の保有等がこれに当たります。治験等の信頼性を確保するため、COIについては、透明性が確保され、科学的な客観性を保証するように適正に管理されることが必要です。

この治験は、名古屋大学からの助成を受けて、また、治験責任医師を含む関係者の研究費を用いて実施されます。また、この治験は、名古屋大学医学部附属病院が、株式会社生命科学インスティテュートから無償提供を受けたCL2020を用いて実施します。この治験で使用するCL2020の生産物賠償責任保険については、株式会社生命科学インスティテュートが負担します。

名古屋大学は、これまでの研究成果を基に、本治験製品の新生児低酸素性虚血性脳症に対する開発に関する特許出願を、株式会社生命科学インスティテュートと共に行っており、治験責任医師を含む関係者が発明者として、将来報酬を得る可能性があります。また、治験責任医師を含む関係者が、本治験以外の研究で株式会社生命科学インスティテュートから共同研究費を受けて、さらに同社のコンサルタントとして業務を引き受けています。ただし、この治験は上記の利益相反について、当院の治験審査委員会（治験審査委員会が必要と判断した場合は、さらに研究利益相反マネジメント委員会）で審査を受け、適正に管理された上で実施されます。また、この治験の結果の解析は、解析計画書に従って実施され、かつ第三者機関による監査を受け、研究の公正性の確保に努めます。そのため、治験の結果について、発明者である治験責任医師を含む関係者や株式会社生命科学インスティテュートに都合の良い結果を意図的に導くことはありません。

## 16. 知的財産権について

この治験の結果として、特許などの<sup>ちてきざいさんけん</sup>知的財産権が生み出される場合もありますが、あなたやあなたのお子さんにはその権利は発生しません。

## <担当医師の連絡先および病院の相談窓口>

治験について何か知りたいことや、何か心配なことがありましたら、担当医師に遠慮なくお尋ねください。

また、治験終了後の結果についてお知りになりたい方は担当医師もしくは病院の相談窓口にご連絡ください。ご連絡いただいた時点で当院が知り得ている情報について説明させていただきます。

●治験責任医師： 総合周産期母子医療センター

講師 佐藤 義朗

●あなたの治験担当医師：所属： \_\_\_\_\_

●連絡先電話番号

名古屋大学医学部附属病院（代表）： \_\_\_\_\_ 内線 \_\_\_\_\_

<夜間・休日のみ>総合周産期母子医療センターNICU： \_\_\_\_\_

担当医師以外の窓口として、先端医療・臨床研究支援センターがあります。何かございましたら、遠慮なくお尋ねください。

●相談窓口： 先端医療・臨床研究支援センター

（名古屋大学医学部附属病院 中央診療棟 A 地下1階）

●担当治験コーディネーター（CRC）： \_\_\_\_\_

●電話番号（直通）： \_\_\_\_\_

<平日 8:30~17:30>

以上、この治験の内容について十分ご理解いただいたうえで、参加していただける場合は、最終ページの同意文書に同意年月日の記載と署名をしてご提出ください。記載していただきました同意文書は3部作成し、あなたが1部、病院が2部それぞれ保管することになります。なお、この説明文書と同意文書（3枚目：患者さん用）を大切に保管しておいてください。

ID番号： \_\_\_\_\_  
 一枚目 カルテ用  (再同意)

## 同意文書

名古屋大学医学部附属病院長 殿

私は、「低体温療法を実施した新生児低酸素性虚血性脳症に対するCL2020の安全性及び忍容性を検討する用量漸増臨床試験」の治験に子供を参加させるにあたり、説明文書を受け取り、その内容について説明を受けました。本治験の内容を十分に理解しましたので、今回の治験に参加することについて私の自由意思にもとづいて同意いたします。なお、いつでも私の意思によって中止できること、中止後も必要かつ可能な治療行為が行われ、病院および治験責任(分担)医師からなんら不利益を受けることがないことを治験責任(分担)医師に確認したため、ここに同意し署名致します。

同意日時：西暦 年 月 日 時 分 代諾者氏名 \_\_\_\_\_ 続柄： \_\_\_\_\_  
 (患者氏名： \_\_\_\_\_)

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_  
 治験責任(分担)医師名 \_\_\_\_\_

説明文書に基づき患児の代諾者に説明を行うとともに、説明文書を手渡しました。

<治験協力者による補助説明時>

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_  
 治験協力者名 \_\_\_\_\_

同意確認および同意文書の手交日時：西暦 年 月 日 時 分

ID番号： —  
二枚目 センター用  (再同意)

## 同意文書

名古屋大学医学部附属病院長 殿

私は、「低体温療法を実施した新生児低酸素性虚血性脳症に対するCL2020の安全性及び忍容性を検討する用量漸増臨床試験」の治験に子供を参加させるにあたり、説明文書を受け取り、その内容について説明を受けました。本治験の内容を十分に理解しましたので、今回の治験に参加することについて私の自由意思にもとづいて同意いたします。なお、いつでも私の意思によって中止できること、中止後も必要かつ可能な治療行為が行われ、病院および治験責任(分担)医師からなんら不利益を受けないことを治験責任(分担)医師に確認したため、ここに同意し署名致します。

同意日時：西暦 年 月 日 時 分 代諾者氏名 \_\_\_\_\_ 続柄： \_\_\_\_\_  
(患者氏名： \_\_\_\_\_)

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_  
治験責任(分担)医師名 \_\_\_\_\_

説明文書に基づき患児の代諾者に説明を行うとともに、説明文書を手渡しました。

<治験協力者による補助説明時>

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_  
治験協力者名 \_\_\_\_\_

同意確認および同意文書の手交日時：西暦 年 月 日 時 分

負担軽減費の受け取りを希望しますか(どちらかに○)： 希望する / 希望しない

「希望する」と答えた方は、以下に振込先および連絡先をご記入ください。

フリガナ							
振込先口座	銀行・信用金庫 農業協同組合					支店	
金融機関コード					支店コード		普通・当座
フリガナ	口座番号						
口座名義	※7桁未満の場合は 右詰で先頭0をつける						
連絡先 (住所・電話 番号)	〒 —						
	TEL： —						



三枚目 患者さん用 □(再同意)

## 同意文書

名古屋大学医学部附属病院長 殿

私は、「低体温療法を実施した新生児低酸素性虚血性脳症に対するCL2020の安全性及び忍容性を検討する用量漸増臨床試験」の治験に子供を参加させるにあたり、説明文書を受け取り、その内容について説明を受けました。本治験の内容を十分に理解しましたので、今回の治験に参加することについて私の自由意思にもとづいて同意いたします。なお、いつでも私の意思によって中止できること、中止後も必要かつ可能な治療行為が行われ、病院および治験責任(分担)医師からなんら不利益を受けることがないことを治験責任(分担)医師に確認したため、ここに同意し署名致します。

同意日時：西暦 年 月 日 時 分 代諾者氏名 \_\_\_\_\_ 続柄： \_\_\_\_\_  
(患者氏名： \_\_\_\_\_)

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_

治験責任(分担)医師名 \_\_\_\_\_

説明文書に基づき患児の代諾者に説明を行うとともに、説明文書を手渡しました。

<治験協力者による補助説明時>

説明日時：西暦 年 月 日 時 分 所属 \_\_\_\_\_

治験協力者名 \_\_\_\_\_

同意確認および同意文書の手交日時：西暦 年 月 日 時 分

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金融機関コード				支店コード			普通・当座
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# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

		Reporting Item	Page Number
<b>Administrative information</b>			
Title	<a href="#">#1</a>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered, name of intended registry	3, 4
Trial registration: data set	<a href="#">#2b</a>	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	<a href="#">#3</a>	Date and version identifier	2
Funding	<a href="#">#4</a>	Sources and types of financial, material, and other support	23, 24
Roles and responsibilities: contributorship	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1, 22, 23

1	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1,2
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	n/a
9	responsibilities:		design; collection, management, analysis, and	
10	sponsor and funder		interpretation of data; writing of the report; and the	
11			decision to submit the report for publication,	
12			including whether they will have ultimate authority	
13			over any of these activities	
14				
15				
16				
17	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	12
18	responsibilities:		coordinating centre, steering committee, endpoint	
19	committees		adjudication committee, data management team,	
20			and other individuals or groups overseeing the trial,	
21			if applicable (see Item 21a for data monitoring	
22			committee)	
23				
24				
25				
26				
27	<b>Introduction</b>			
28				
29				
30	Background and	<a href="#">#6a</a>	Description of research question and justification for	5
31	rationale		undertaking the trial, including summary of relevant	
32			studies (published and unpublished) examining	
33			benefits and harms for each intervention	
34				
35				
36	Background and	<a href="#">#6b</a>	Explanation for choice of comparators	n/a; This is a
37	rationale: choice of			single-arm,
38	comparators			dose-
39				escalation
40				trial.
41				
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44				
45	Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	9
46				
47	Trial design	<a href="#">#8</a>	Description of trial design including type of trial (eg,	9
48			parallel group, crossover, factorial, single group),	
49			allocation ratio, and framework (eg, superiority,	
50			equivalence, non-inferiority, exploratory)	
51				
52				
53				
54	<b>Methods:</b>			
55	<b>Participants,</b>			
56	<b>interventions, and</b>			
57	<b>outcomes</b>			
58				
59				
60				

1	Study setting	<a href="#">#9</a>	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
2				
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8	Eligibility criteria	<a href="#">#10</a>	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, 11
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14	Interventions: description	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
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20	Interventions: modifications	<a href="#">#11b</a>	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	n/a
21				
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27	Interventions: adherence	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
28				
29				
30				
31				
32	Interventions: concomitant care	<a href="#">#11d</a>	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
33				
34				
35				
36	Outcomes	<a href="#">#12</a>	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16
37				
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47				
48	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 1
49				
50				
51				
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54				
55	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve study objectives and how it was determined,	17
56				
57				
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60				

including clinical and statistical assumptions supporting any sample size calculations

Recruitment [#15](#) Strategies for achieving adequate participant enrolment to reach target sample size 9, 10

## Methods:

### Assignment of interventions (for controlled trials)

Allocation: sequence generation [#16a](#) Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions n/a; This study will be open label.

Allocation concealment mechanism [#16b](#) Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned n/a; This study will be open label.

Allocation: implementation [#16c](#) Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions n/a; This study will be open label.

Blinding (masking) [#17a](#) Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how n/a; This study will be open label.

Blinding (masking): emergency unblinding [#17b](#) If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a; This study will be open label.

### Methods: Data collection, management, and analysis

Data collection plan [#18a](#) Plans for assessment and collection of outcome, baseline, and other trial data, including any related 14

processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol

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10	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete
11	retention		follow-up, including list of any outcome data to be
12			collected for participants who discontinue or deviate
13			from intervention protocols
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17	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,
18			including any related processes to promote data
19			quality (eg, double data entry; range checks for data
20			values). Reference to where details of data
21			management procedures can be found, if not in the
22			protocol
23			
24			
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26	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and
27			secondary outcomes. Reference to where other
28			details of the statistical analysis plan can be found, if
29			not in the protocol
30			
31			
32			
33	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup
34	analyses		and adjusted analyses)
35			
36			
37	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol
38	population and		non-adherence (eg, as randomised analysis), and
39	missing data		any statistical methods to handle missing data (eg,
40			multiple imputation)
41			
42			
43			
44	<b>Methods:</b>		
45	<b>Monitoring</b>		
46			
47			
48	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC);
49	formal committee		summary of its role and reporting structure;
50			statement of whether it is independent from the
51			sponsor and competing interests; and reference to
52			where further details about its charter can be found,
53			if not in the protocol. Alternatively, an explanation of
54			why a DMC is not needed
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1	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping	n/a
2	interim analysis		guidelines, including who will have access to these	
3			interim results and make the final decision to	
4			terminate the trial	
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8	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and	12,16
9			managing solicited and spontaneously reported	
10			adverse events and other unintended effects of trial	
11			interventions or trial conduct	
12				
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14	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct,	18
15			if any, and whether the process will be independent	
16			from investigators and the sponsor	
17				
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20	<b>Ethics and</b>			
21	<b>dissemination</b>			
22				
23				
24	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /	19
25	approval		institutional review board (REC / IRB) approval	
26				
27				
28	Protocol	<a href="#">#25</a>	Plans for communicating important protocol	19
29	amendments		modifications (eg, changes to eligibility criteria,	
30			outcomes, analyses) to relevant parties (eg,	
31			investigators, REC / IRBs, trial participants, trial	
32			registries, journals, regulators)	
33				
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36	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	19, 20
37			potential trial participants or authorised surrogates,	
38			and how (see Item 32)	
39				
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41	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use	n/a
42	ancillary studies		of participant data and biological specimens in	
43			ancillary studies, if applicable	
44				
45				
46	Confidentiality	<a href="#">#27</a>	How personal information about potential and	20
47			enrolled participants will be collected, shared, and	
48			maintained in order to protect confidentiality before,	
49			during, and after the trial	
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53	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	24
54	interests		investigators for the overall trial and each study site	
55				
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1	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
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6	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
7	trial care			
8				
9				
10				
11	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	20, 24
12	trial results			
13				
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20				
21	Dissemination policy:	<a href="#">#31b</a>	Authorship eligibility guidelines and any intended use of professional writers	n/a;
22	authorship			Authorship
23				eligibilities
24				were
25				confirmed by
26				standard
27				material.
28				
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31				
32	Dissemination policy:	<a href="#">#31c</a>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
33	reproducible			
34	research			
35				
36				
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38	<b>Appendices</b>			
39				
40	Informed consent	<a href="#">#32</a>	Model consent form and other related documentation given to participants and authorised surrogates	n/a
41	materials			
42				
43				
44				
45	Biological specimens	<a href="#">#33</a>	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a
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