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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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Keywords

Hypoxic-ischemic encephalopathy, neonates, cerebral palsy, hypothermia, mesenchymal stem cell, Muse cell

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Abstract

Introduction Neonatal hypoxic-ischemic encephalopathy (HIE) is an important illness associated with death or cerebral palsy. This study aims to assess the

safety and tolerability of the allogenic human multilineage-differentiating stress-enduring cell (Muse cell)-based product, CL2020, in newborns with HIE. This is the first clinical trial of CL2020 for neonates.

Methods and analysis This is a single-center, open-label, dose-escalation study enrolling up to 12 patients. Neonates with HIE who receive proper hypothermia therapy will be included in this study. A single intravenous injection of CL2020 will be administered between 5 and 14 days of age. Subjects in the low-dose and high-dose cohorts will receive 1.5 million cells and 15 million cells per dose, respectively. The primary outcome is the incidence of any adverse events until 12 weeks after administration. The main secondary outcome is the Bayley Scales of Infant and Toddler Development Third Edition score and the developmental quotient as per the Kyoto Scale of Psychological Development 2001 at 78 weeks.

Ethics and dissemination This study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The Nagoya University Hospital Institutional Review Board (No. 312005) approved this study on November 13, 2019.

Trial registration ClinicalTrials.gov: NCT04261335, registered on 7 February

2020, https://clinicaltrials.gov/ct2/show/NCT04261335. Japan Registry of Clinical Trials: jRCT2043190112, registered on 6 February 2020, https://jrct.niph.go.jp/latest-detail/jRCT2043190112

Strengths and limitations of this study

- This is the first clinical trial aiming at the safety and tolerability of CL2020, a Muse cell-based product, in neonates.
- Hypothermia is currently the sole neuroprotective therapy for HIE; however, its effectiveness is insufficient, and a novel therapy is therefore required.
- After confirming the safety and tolerability of intravenous CL2020 administration in neonates, we will need a randomized placebo-controlled clinical trial to evaluate the effectiveness of CL2020 for HIE.
- This clinical trial is the first clinical application in neonates based on our non-clinical study results; if this product is safe and well-tolerated by neonates in this study, this may expand its application to other disorders in neonates and children.

Introduction

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

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

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

HIE. Preclinical animal studies using umbilical cord blood cells (UCBCs) in neonatal HIE and stroke rat models have reported effectiveness.[7–9] In addition, some exploratory clinical studies have shown the safety and feasibility of autologous UCBCs administration for HIE neonates.[10, 11] However, the preparation of autologous UCBCs requires well-equipped facilities and sufficient human resources in birthing centers, clinics, or hospitals.

From a wide variety of options as candidates for regenerative cells,[12–15] we have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent markers, that self-renew and differentiate from a single cell into each of the three germ layer cells.[16] They are positive for both stage-specific embryonic antigen (SSEA)-3 and CD105 in the peripheral blood, bone marrow, and organ connective tissues.[17, 18] Muse cells also have a specific immunomodulatory system, represented by human leukocyte antigen (HLA) -G expression, allowing them to be directly administered without HLA matching or immunosuppressant agents.[19] Furthermore, after intravenous administration, Muse cells are distributed to the damaged site by sphingosine monophosphate (S1P)-sphingosine

monophosphate receptor 2 (S1PR2) axis mechanism,[19] and then self-renewed without artificial differentiation or induction. After migrating, Muse cells differentiate into tissue-compatible cells according to the microenvironment and remain integrated in the host tissue to participate in tissue repair.[20, 21] Based on these characteristics, intravenous administration of allogenic Muse cells are expected to be an effective regenerative therapy for HIE.

We found that the systemic administration of human Muse cells for the perinatal HIE rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left carotid artery, improved learning deficits and motor impairment. In addition, human Muse cells are localized in the damaged brain and differentiate into neurons. These effects were much clearer in the Muse cells than in MSCs without Muse cells subpopulation.[22] Moreover, we confirmed that the human allogenic Muse cells-based product, CL2020, manufactured by Life Science Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical Holdings Corporation, exerted a treatment effect with no toxicity in the HIE rat models. To verify the safety and effectiveness of CL2020, LSII has conducted several clinical trials with adult patients, namely, acute myocardial infarction (JapicCTI-No.:

JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103), epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841), amyotrophic lateral sclerosis (jRCT2063200047), and acute respiratory distress syndrome associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-human clinical trial for acute myocardial infarction was performed in 3 patients and indicated that CL2020 was safe and significantly improved the left ventricular ejection fraction.[23] A phase 1/2 open-label study for adult epidermolysis bullosa was also recently published. Five patients received a single injection of CL2020, and the ulcer size was significantly reduced for up to 3 months.[24] Nevertheless, the safety and tolerance of Muse cells in neonates are unknown because they have never been administered to neonates. Based on these results, we planned the first-in-neonate clinical trial to confirm the safety and tolerability of CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy. Hence, we describe the detailed design of an investigator-initiated clinical trial on neonatal HIE to investigate the safety, tolerability, and efficacy in neurodevelopmental outcomes at 18 months. This clinical trial was named "The Evaluation of Safety and Tolerability of CL2020 in Neonatal Hypoxic Ischemic

Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation Clinical Trial" (the SHIELD trial).

Methods and analysis

Objective and study design

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

Recruitment and setting

Patient recruitment is performed in our hospital or by receiving referrals of patients from other hospitals in our district. Prior to the screening assessment, the investigators will obtain written informed consent from the patients' legal parental authority. After conducting the screening assessment and verifying the patients'

eligibility, they will be registered for the trial.

Participants

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

1) At least 36 weeks gestational age, and one of the following criteria (i –iii.)
   i. Apgar score ≤ 5 at 10 minutes
   ii. Continued neonatal resuscitation for at least 10 minutes
   iii. pH < 7.0, or base deficit ≥ 16 mmol/L in any blood sample obtained within 60 min after birth
2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]
3) Undergone therapeutic hypothermia started within 6 hours after birth and continued for 72 hours
4) Birth weight ≥ 1,800 g
5) Heart rate ≥ 100/ min, and SpO_2 ≥ 90%
6) Able to provide voluntary informed consent after receiving information about the study (consent will be obtained from a legal proxy).

Participants will be excluded according to the following exclusion criteria:

1) Suspected or confirmed severe congenital abnormalities or chromosomal anomaly
2) Planned to undergo surgery or radiation therapy
3) Scheduled to take systemic corticosteroids treatment for over 5 days
4) Blood glucose ≥ 200 mg/dL continuously sustained
5) Participation in another interventional clinical study
6) Suspected or confirmed active and severe infection
7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or syphilis serum reaction
8) History of severe hypersensitivity or anaphylactic reaction
9) Severe complications

Patient and public involvement

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

Intervention and follow-up

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

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

Table 1

Schedule of interventions and assessments

Treatments and assessments	Registration	Day 0	Day 1	Day 3	Week 1	Week 2	Week 4	Week 12	Week 26	Week 38	Week 52	Week 78
Agreement	x											
Demographics, current medications	x											
Registration	x											
Assignment	x											
Administration		x										
Hospitalization		←	—	—	—	→						
Vital signs[a]	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[b]	x	x	x	x	x	x	x	x	x		x	x
Biochemical tests[c]	x	x	x	x	x	x	x	x	x		x	x
Urine analysis[d]	x	x	x		x	x						
Composite endpoints		←	—	—	—	—	—	—	—	—	—	→
Spasticity								x	x	x	x	x
Postnatal development									x	x	x	x
Epilepsy						←	—	—	—	—	—	→
MRI	x					x						x

Bayley scale[e]		x
Kyoto scale[f]		x
GMFCS[g]		x

[a]Blood pressure, pulse rate, and body temperature

[b]Red bood cell count, hemoglobin, hematocrit, white blood cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monospheres), and platelet count

[c]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.

[d]pH, urine protein, urine occult blood, and urine sugar

[e]Bayley Scales of Infant and Toddler Development Third edition

[f]Kyoto Scale of Psychological Development 2001

[g]Expanded and Revised Gross Motor Function Classification System

Study endpoints

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

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

7) Assessment of postnatal development such as head control, rolling, sitting, crawling, walking unaided, and saying several meaningful words

8) Presence of spasticity: The definition of spasticity is the status of increased muscle tone or increased deep tendon reflex

9) Presence of epilepsy: The definition of epilepsy is based on the International League Against Epilepsy[29]

10) Magnetic resonance imaging score: The scoring system is based on the report of Barkovich et al.[30]

11) The score of Expanded and Revised Gross Motor Function Classification System[31] at 78 weeks

In addition, we will collect vital signs and laboratory values for safety assessment at specific points, as shown in Table 1.

Sample size calculation

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

Statistical analysis

All analyses are based on an intention-to-treat principle. All adverse events will be confirmed for the primary endpoint, and the proportions of the adverse events and their 95% CI based on the Clopper-Pearson method will be calculated. Time-to-event data will be summarized using the Kaplan-Meier method. Descriptive statistics for continuous variables and frequency and proportion for categorical variables will be calculated for each secondary endpoint. Statistical analysis will be performed using SAS software (SAS Institute, version 9.4, North Carolina, USA). Statistical significance will be defined as $p < 0.05$.

Monitoring and auditing

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

Status of this trial

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

Ethics and dissemination

Ethics approval

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

Patient consent for participation

The investigators and trained clinical research coordinators will introduce the trial to patients' legal representatives with prepared information sheets and informed consent forms. The investigator will obtain written consent to participate in the trial. Identification of all subjects during the data collection will be performed using a subject identification code, and all personnel involved in this study will take the best possible precautions to ensure the protection of patients' personal information.

Dissemination

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

Discussion

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

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

Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy. Several randomized control trials of hypothermia therapy for HIE have been conducted,[32–37] and hypothermia is currently the sole neuroprotective therapy. However, its effectiveness is insufficient; therefore, a novel therapy is required. Regenerative therapy is the focus of next-generation therapy. Clinical studies with autologous UCBCs for HIE had been conducted before the development of CL2020.[10, 11] This UCBCs therapy requires additional equipment and human resources for its preparation because the newborns' umbilical cord blood has to be collected at birth, and the patients receive the first dose of prepared UCBCs within 24 hours after birth. In contrast, in our non-clinical study, single intravenous administration of Muse cells for HIE model rats 3 days after hypoxic-ischemic injury ameliorated behavioral abnormalities up to 5 months.[22] In a non-clinical study using CL2020, the treatment effect was exerted at even later administration timing. Thus, we set the administration of Muse cells to human neonates between 5 and 14 days after birth, which means that physicians and patients' families can afford the time to decide or prepare

the treatment based on patient condition or seek further opinions.

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

Herein, we present the overall design of this single-center, open-label, dose-escalation clinical trial of Muse cell products in HIE patients with hypothermia. This clinical trial is the first clinical application of CL2020 in neonates based on our non-clinical study results, and if we can verify the safety and well-tolerability of this product in neonates, it may expand its application to other disorders in neonates and children.

List of abbreviations

CI, confidence interval; DSMB, data and safety monitoring board; GMFCS: expanded and revised gross motor function classification system; HIE, hypoxic-ischemic encephalopathy; HLA, human leukocyte antigen; MSC, mesenchymal

stem cell; Muse cell, multilineage-differentiating stress-enduring cell; UCBC, umbilical cord blood cell

Authors' contributions

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

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Competing interests statement

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

Data availability statement

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

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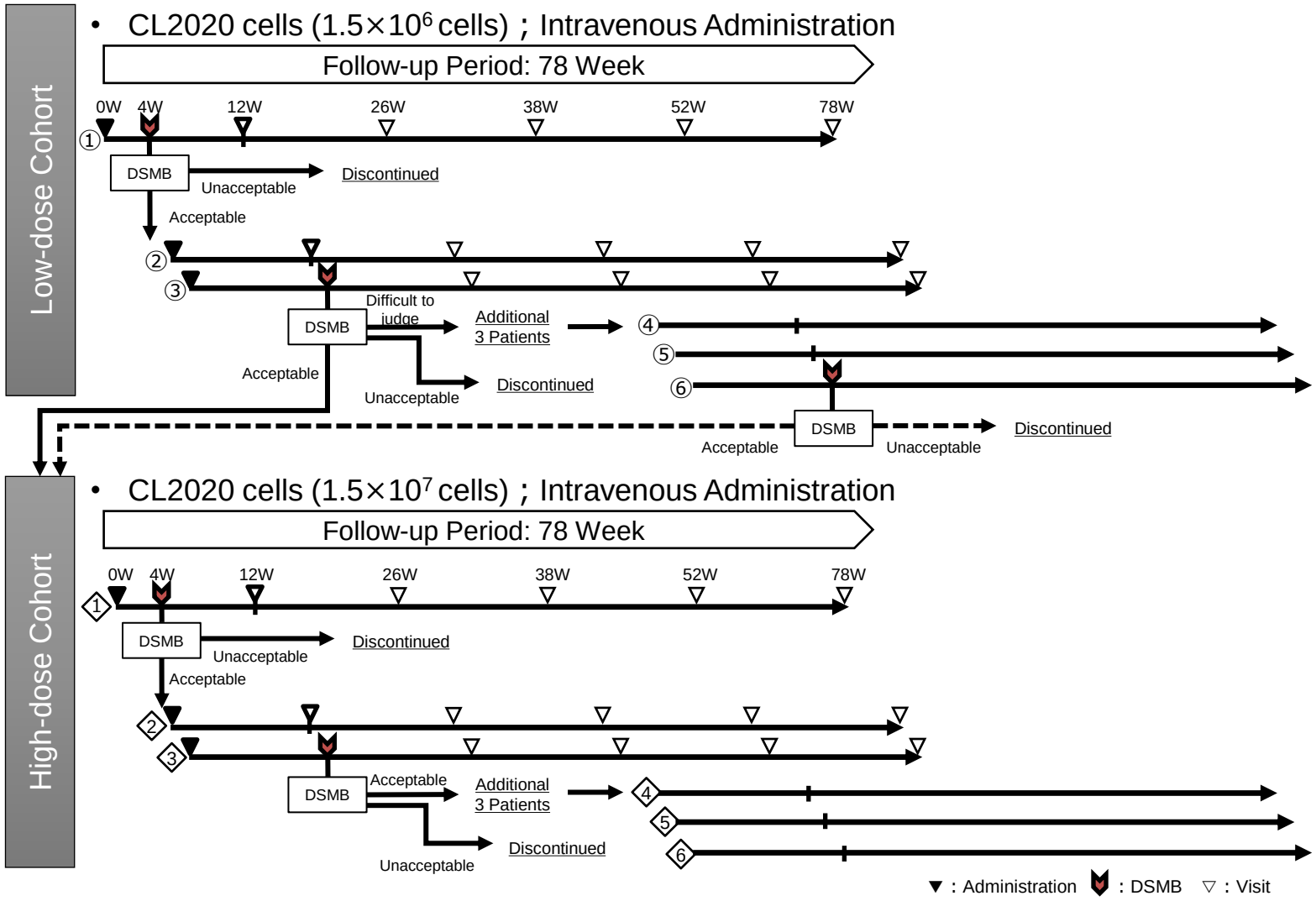

Figure 1 Study framework

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.

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

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1,2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a; This is a single-arm, dose-escalation trial.
Objectives	#7	Specific objectives or hypotheses	9
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
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Study setting	#9	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
Eligibility criteria	#10	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
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
Interventions: modifications	#11b	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
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	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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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	
Data collection plan: retention	#18b	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
Data management	#19	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
Statistics: outcomes	#20a	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
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
Statistics: analysis population and missing data	#20c	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			
Data monitoring: formal committee	#21a	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

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12,16
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Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	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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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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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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Abstract

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

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

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

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

Trial registration: ClinicalTrials.gov: NCT04261335, registered on 7 February 2020, https://clinicaltrials.gov/ct2/show/NCT04261335. Japan Registry of Clinical Trials: jRCT2043190112, registered on 6 February 2020, https://jrct.niph.go.jp/latest-detail/jRCT2043190112

Strengths and limitations of this study

- This is the first clinical trial aimed at the safety and tolerability of CL2020, a Muse cell-based product, in neonates.
- Hypothermia is currently the sole neuroprotective therapy for HIE; its effectiveness is insufficient, and a novel therapy is required.
- After confirming the safety and tolerability of intravenous CL2020 in neonates, we will need a randomised placebo-controlled clinical trial to evaluate the effectiveness of CL2020 for treating HIE.
- This trial is the first clinical application in neonates based on our non-clinical study results; if this product is found to be safe and well-tolerated by neonates, its

application may expand to other disorders in neonates and children.

Keywords

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

INTRODUCTION

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

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

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

From a wide variety of options as candidates for regenerative cells,[12-15] we have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent markers that self-renew and differentiate from a single cell into each of the three

germ layer cells.[16] They are positive for stage-specific embryonic antigen (SSEA)-3 and CD105 in the peripheral blood, bone marrow, and organ connective tissues.[17, 18] Muse cells also have a specific immunomodulatory system, represented by human leukocyte antigen (HLA) -G expression, allowing them to be directly administered without HLA matching or immunosuppressant agents.[19] Furthermore, after intravenous administration, Muse cells are distributed to the damaged site by sphingosine monophosphate (S1P)-sphingosine monophosphate receptor 2 (S1PR2) axis mechanism,[19] and then self-renewed without artificial differentiation or induction. After migrating, Muse cells differentiate into tissue-compatible cells according to the microenvironment and remain integrated into the host tissue to participate in tissue repair.[20, 21] Based on these characteristics, intravenous administration of allogenic Muse cells is expected to be an effective regenerative therapy for HIE.

We found that the systemic administration of human Muse cells in the perinatal HIE rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left carotid artery, improved learning deficits and motor impairment. In addition, human Muse cells are localised in the damaged brain and differentiate into neurons.

These effects were much clearer in the Muse cells than in mesenchymal stem cells (MSCs) without Muse cells subpopulation.[22] Moreover, we confirmed that the human allogenic Muse cells-based product, CL2020, manufactured by Life Science Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical Holdings Corporation, exerted a therapeutic effect with no toxicity in the HIE rat models. To verify the safety and effectiveness of CL2020, LSII has conducted several clinical trials in adult patients with acute myocardial infarction (JapicCTI-No.: JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103), epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841), amyotrophic lateral sclerosis (jRCT2063200047), and acute respiratory distress syndrome associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-human clinical trial for acute myocardial infarction was performed in 3 patients and indicated that CL2020 was safe and significantly improved the left ventricular ejection fraction.[23] A phase 1/2 open-label trial on adult epidermolysis bullosa was also recently published. A total of 5 patients received a single injection of CL2020, and the ulcer size was significantly reduced for up to 3 months.[24] Nevertheless, the safety and tolerability of Muse cells in neonates are unknown

because they have never been administered to neonates. Based on these results, we planned the first-in-neonate clinical trial to confirm the safety and tolerability of CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy. Hence, we describe the detailed design of an investigator-initiated clinical trial on neonatal HIE to investigate the safety, tolerability, and efficacy in neurodevelopmental outcomes at 18 months. This clinical trial is named "The Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring cell-based product in Neonatal Hypoxic-Ischeamic Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation Clinical Trial" (the SHIELD trial).

METHODS AND ANALYSIS

Objective and study design

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

administering CL2020 to each patient.

Recruitment and setting

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

Participants

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

1) At least 36 weeks gestational age, and one of the following criteria (i–iii)
   i. Apgar score ≤5 at 10 minutes
   ii. Continued neonatal resuscitation for at least 10 minutes
   iii. pH <7.0, or base deficit ≥16 mmol/L in any blood sample obtained within 60 min after birth
2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]

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

4) Birth weight ≥1,800 g

5) Heart rate ≥100/min, and SpO_2 ≥90%

6) Able to provide voluntary informed consent after receiving information about the study (consent will be obtained from a legal proxy).

Exclusion criteria are:

1) Suspected or confirmed severe congenital abnormalities or chromosomal anomaly

2) Planned to undergo surgery or radiation therapy

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

4) Blood glucose ≥200 mg/dL continuously sustained

5) Participation in another interventional clinical study

6) Suspected or confirmed active and severe infection

7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or syphilis serum reaction

8) History of severe hypersensitivity or anaphylactic reaction

9) Severe complications not related to HIE

Patient and public involvement

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

Intervention and follow-up

The clinical-grade Muse cell-based product, CL2020 (1.5×10^7 cells/15 mL of frozen preparation), was produced from human allogenic MSCs by LSII.[26] The CL2020 was produced by exposing MSCs to some stressors, and they were enriched to be positive for both SSEA3 and CD105 but negative for CD45. We will prepare cells from CL2020 for administration to neonates by centrifuging the product after thawing, removing the supernatant, and suspending with acetated Ringer's solution as 15 million cells in 15 mL. The patients will receive the prepared cells intravenously once between 5 and 14 days after birth. This study will utilise a 3 + 3 dose-escalation design, setting two cohorts for the injected dose. Subjects in the low-dose cohort will receive 1.5 million cells in 1.5 mL for 2 min, while those in the high-dose cohort will

receive 15 million cells in 15 mL for 20 minutes. The following treatments will be prohibited during the study: corticosteroids (prednisolone converted at 2 mg/kg/day or more, and more than 5 days), other human MSC products, processed cell products except for the red blood cells, other investigational products, and the use of investigational medical devices. The data and safety monitoring board (DSMB) will consist of 3 specialists in paediatric and perinatal care independent of the trial investigators. The DSMB will be held at predefined times in both cohorts: at 4 weeks after administering to the first patient and 12 weeks after administering to the third patient in each cohort. The council will also be held when a product-related severe adverse event occurs or when investigators consider that it should be convened due to safety concerns. The DSMB will recommend whether this trial should be moved forward or be discontinued. Figure 1 illustrates the framework of this study. The study participants will be hospitalised for at least 2 weeks after CL2020 administration and followed up for 78 weeks. The planned visits and data collection are presented in Table 1.

Table 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											
Demographics, current medications	x											
Registration	x											
Assignment	x											
Administration		x										
Hospitalisation		←	—	—	—	—	→					
Vital signs[a]	x	x	x	x	x	x	X	x	x	x	x	x
Oxygen saturation	x	x	x	x	x	x	X	x	x	x	x	x
Haematological tests[b]	x	x	x	x	x	x	X	x	x		x	x
Biochemical tests[c]	x	x	x	x	x	x	X	x	x		x	x
Urine analysis[d]	x	x	x		x	x						
Composite endpoints		←	—	—	—	—	—	—	—	—	—	→
Spasticity								x	x	x	x	x
Postnatal development									x	x	x	x

Schedule of interventions and assessments

15

Epilepsy		←	→
MRI	x	x	x
Bayley scale[e]			x
Kyoto scale[f]			x
GMFCS[g]			x

[a]Blood pressure, pulse rate, and body temperature

[b]Red blood cell count, haemoglobin, haematocrit, white blood cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monocytes), and platelet count

[c]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.

[d]pH, urine protein, urine occult blood, and urine sugar

[e]Bayley Scales of Infant and Toddler Development Third edition

[f]Kyoto Scale of Psychological Development 2001

[g]Expanded and Revised Gross Motor Function Classification System

Study endpoints

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

1) Incidence of composite endpoints (death, continuous respiratory support, or continuous use of vasopressors or pulmonary vasodilators)
2) Mortality and overall survival
3) Duration of continuous respiratory support: The definition of respiratory support is the status of conducting artificial ventilation with tracheal intubation.
4) Duration of continuous use of vasopressors or pulmonary vasodilators: dopamine, dobutamine, adrenaline, noradrenaline, milrinone, vasopressin, *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide, epoprostenol sodium, nitroglycerin, and alprostadil alfadex
5) The Bayley Scales of Infant and Toddler Development Third Edition [27] score at 78 weeks
6) Developmental quotient as per the Kyoto Scale of Psychological Development 2001[28] at 78 weeks
7) Assessment of postnatal development such as head control, rolling, sitting, crawling, unaided walking, and saying several meaningful words

8) Presence of spasticity: The definition of spasticity is the status of increased muscle tone or increased deep tendon reflex.

9) Presence of epilepsy: The definition of epilepsy is based on the International League Against Epilepsy.[29]

10) Magnetic resonance imaging score: The scoring system is based on the report of Barkovich et al.[30]

11) The score of Expanded and Revised Gross Motor Function Classification System[31] at 78 weeks

In addition, we will collect vital signs and laboratory values for safety assessment at specific points, as shown in Table 1.

Sample size calculation

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

Statistical analysis

All analyses are based on an intention-to-treat principle. We will analyse adverse

events on the safety analysis set defined as all subjects enrolled in this study and received the investigational cell product. All adverse events will be confirmed for the primary endpoint, and the proportions of the adverse events and their 95% CI based on the Clopper-Pearson method will be calculated. Overall survival, defined as the time from birth to the date of death due to any cause, will be summarised using the Kaplan-Meier method. Descriptive statistics for continuous variables and frequency and proportion for categorical variables will be calculated for each secondary endpoint. Statistical analysis will be performed using the SAS software (SAS Institute, version 9.4, North Carolina, USA). Statistical significance will be defined as p <0.05.

Monitoring and auditing

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

Status of this trial

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

ETHICS AND DISSEMINATION

Ethical approval

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

Patient consent for participation

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

Dissemination

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

DISCUSSION

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

trials of CL2020, we decided to implement this clinical trial to ensure safety in neonates.

Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy. Several randomised controlled trials of hypothermia therapy for HIE have been conducted,[32-37] and hypothermia is currently the sole neuroprotective therapy. However, its effectiveness is insufficient, and a novel therapy is required. Regenerative therapy is the focus of next-generation therapy. Clinical studies with autologous UCBCs for HIE had been conducted before the development of CL2020.[10, 11] This UCBCs therapy requires additional equipment and human resources for its preparation because the newborns' umbilical cord blood has to be collected at birth, and the patients receive the first dose of prepared UCBCs within 24 hours after birth. In contrast, in our non-clinical study, single intravenous administration of Muse cells to HIE model rats 3 days after hypoxic-ischaemic injury ameliorated behavioural abnormalities up to 5 months.[22] In a non-clinical study using CL2020, the treatment effect was exerted at even 7 days after insult by hypoxic ischaemia. In addition, a single dose of CL2020 administered via the vein at the subacute (about 9 days after onset) and chronic phases (about 30 days) was effective in a mouse lacunar stroke model.[26] Thus, we set the

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

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

Herein, we present the overall design of this single-centre, open-label, dose-escalation clinical trial of Muse cell products in HIE patients with hypothermia. This clinical trial is the first clinical application of CL2020 in neonates based on our non-clinical study results. If we can verify that this product is safe and well-tolerable in neonates, its application may expand to other disorders in neonates and children.

Funding

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Contributorship Statement

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

the manuscript. SSh and YS have revised the manuscript. All authors read and approved the final manuscript.

Competing Interests

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

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Data availability statement

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

Figure 1

Study framework

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

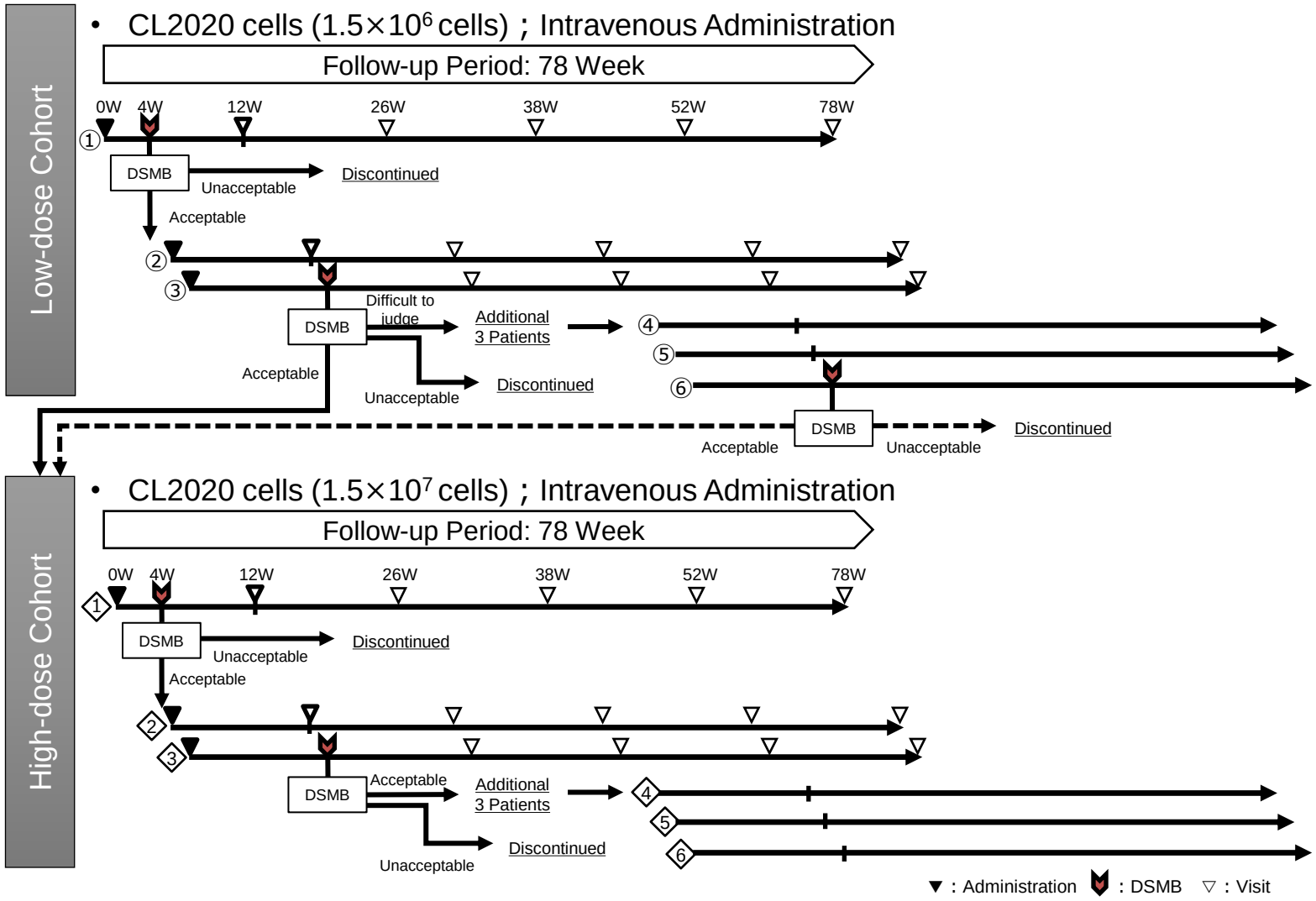

Figure 1 Study framework

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.

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

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

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1,2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a; This is a single-arm, dose-escalation trial.
Objectives	#7	Specific objectives or hypotheses	9
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			

Study setting	#9	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
Eligibility criteria	#10	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
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
Interventions: modifications	#11b	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
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	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
Participant timeline	#13	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
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined,	17

		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	
Data collection plan: retention	#18b	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
Data management	#19	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
Statistics: outcomes	#20a	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
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
Statistics: analysis population and missing data	#20c	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			
Data monitoring: formal committee	#21a	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

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12,16
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	19
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19, 20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	20
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	24

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	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
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a; Authorship eligibilities were confirmed by standard material.
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	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

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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Primary Subject Heading:	Paediatrics
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Keywords:	NEONATOLOGY, Paediatric intensive & critical care < INTENSIVE & CRITICAL CARE, Clinical trials < THERAPEUTICS

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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Word count 2,763 words

Abstract

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

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

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

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

Trial registration: ClinicalTrials.gov: NCT04261335, registered on 7 February 2020, https://clinicaltrials.gov/ct2/show/NCT04261335. Japan Registry of Clinical Trials: jRCT2043190112, registered on 6 February 2020, https://jrct.niph.go.jp/latest-detail/jRCT2043190112

Strengths and limitations of this study

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

Keywords

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

INTRODUCTION

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

The most evidence-based treatment for moderate-to-severe HIE is therapeutic

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

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

From a wide variety of options as candidates for regenerative cells,[12-15] we have noted the multilineage-differentiating stress-enduring cells (Muse cells). Muse cells are endogenous, non-tumorigenic, pluripotent-like stem cells positive for pluripotent markers that self-renew and differentiate from a single cell into each of the three germ layer cells.[16] They are positive for stage-specific embryonic antigen (SSEA)-

3 and CD105 in the peripheral blood, bone marrow, and organ connective tissues.[17, 18] Muse cells also have a specific immunomodulatory system, represented by human leukocyte antigen (HLA) -G expression, allowing them to be directly administered without HLA matching or immunosuppressant agents.[19] Furthermore, after intravenous administration, Muse cells are distributed to the damaged site by sphingosine monophosphate (S1P)-sphingosine monophosphate receptor 2 (S1PR2) axis mechanism,[19] and then self-renewed without artificial differentiation or induction. After migrating, Muse cells differentiate into tissue-compatible cells according to the microenvironment and remain integrated into the host tissue to participate in tissue repair.[20, 21] Based on these characteristics, intravenous administration of allogenic Muse cells is expected to be an effective regenerative therapy for HIE.

We found that the systemic administration of human Muse cells in the perinatal HIE rat model, made by 60 min of hypoxic (8%) exposure following ligation of the left carotid artery, improved learning deficits and motor impairment. In addition, human Muse cells are localised in the damaged brain and differentiate into neurons. These effects were much clearer in the Muse cells than in mesenchymal stem cells

(MSCs) without Muse cells subpopulation.[22] Moreover, we confirmed that the human allogenic Muse cells-based product, CL2020, manufactured by Life Science Institute, Inc. (LSII; Tokyo, Japan), a group company of the Mitsubishi Chemical Holdings Corporation, exerted a therapeutic effect with no toxicity in the HIE rat models. To verify the safety and effectiveness of CL2020, LSII has conducted several clinical trials in adult patients with acute myocardial infarction (JapicCTI-No.: JapicCTI-183834 and JapicCTI-195067), stroke (JapicCTI-184103), epidermolysis bullosa (JapicCTI-184563), spinal cord injury (JapicCTI-194841), amyotrophic lateral sclerosis (jRCT2063200047), and acute respiratory distress syndrome associated with SARS-CoV-2 infection (jRCT2043210005). The first-in-human clinical trial for acute myocardial infarction was performed in 3 patients and indicated that CL2020 was safe and significantly improved the left ventricular ejection fraction.[23] A phase 1/2 open-label trial on adult epidermolysis bullosa was also recently published. A total of 5 patients received a single injection of CL2020, and the ulcer size was significantly reduced for up to 3 months.[24] Nevertheless, the safety and tolerability of Muse cells in neonates are unknown because they have never been administered to neonates. Based on these results,

we planned the first-in-neonate clinical trial to confirm the safety and tolerability of CL2020 in patients with moderate-to-severe HIE receiving hypothermia therapy. Hence, we describe the detailed design of an investigator-initiated clinical trial on neonatal HIE to investigate the safety, tolerability, and efficacy in neurodevelopmental outcomes at 18 months. This clinical trial is named "The Evaluation of Safety and Tolerability of a multilineage-differentiating stress-enduring cell-based product in Neonatal Hypoxic-Ischeamic Encephalopathy Patients with Therapeutic Hypothermia in the Dose Escalation Clinical Trial" (the SHIELD trial).

METHODS AND ANALYSIS

Objective and study design

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

Recruitment and setting

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

Participants

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

1) At least 36 weeks gestational age, and one of the following criteria (i–iii)
   - i. Apgar score ≤5 at 10 minutes
   - ii. Continued neonatal resuscitation for at least 10 minutes
   - iii. pH <7.0, or base deficit ≥16 mmol/L in any blood sample obtained within 60 min after birth
2) Moderate or severe encephalopathy, as judged using the Sarnat criteria[25]

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

4) Birth weight ≥1,800 g

5) Heart rate ≥100/min, and SpO_2 ≥90% upon enrollment

6) Able to provide voluntary informed consent after receiving information about the study (consent will be obtained from a legal proxy).

Exclusion criteria are:

1) Suspected or confirmed severe congenital abnormalities or chromosomal anomaly

2) Planned to undergo surgery or radiation therapy

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

4) Blood glucose ≥200 mg/dL continuously sustained

5) Participation in another interventional clinical study

6) Suspected or confirmed active and severe infection

7) Positive for HBs antigen, HCV antibody, HIV antibody, HTLV-1 antibody, or syphilis serum reaction

8) History of severe hypersensitivity or anaphylactic reaction

9) Severe complications not related to HIE

Patient and public involvement

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

Intervention and follow-up

The clinical-grade Muse cell-based product, CL2020 (1.5×10^7 cells/15 mL of frozen preparation), was produced from human allogenic MSCs by LSII.[26] The CL2020 was produced by exposing MSCs to some stressors, and they were enriched to be positive for both SSEA3 and CD105 but negative for CD45. We will prepare cells from CL2020 for administration to neonates by centrifuging the product after thawing, removing the supernatant, and suspending with acetated Ringer's solution. The patients will receive the prepared cells intravenously once between 5 and 14 days after birth. We decided to administer as soon as possible within this window (5–14 days after birth) in principle after registration. This study will utilise a 3 + 3 dose-

escalation design, setting two cohorts for the injected dose. Subjects in the low-dose cohort will receive 1.5 million cells, while those in the high-dose cohort will receive 15 million cells. The following treatments will be prohibited during the study: corticosteroids (prednisolone converted at 2 mg/kg/day or more, and more than 5 days), other human MSC products, processed cell products except for the red blood cells, other investigational products, and the use of investigational medical devices. Regarding corticosteroid, it affects cell proliferation mediated by RNA transcription [27], we thought that they could affect the function of the administered cells. The data and safety monitoring board (DSMB) will consist of 3 specialists in paediatric and perinatal care independent of the trial investigators. The DSMB will be held at predefined times in both cohorts: at 4 weeks after administering to the first patient and 12 weeks after administering to the third patient in each cohort. The council will also be held when a product-related severe adverse event occurs or when investigators consider that it should be convened due to safety concerns. The DSMB will recommend whether this trial should be moved forward or be discontinued. Figure 1 illustrates the framework of this study. The study participants will be hospitalised for at least 2 weeks after CL2020 administration and followed up for 78

weeks. The planned visits and data collection are presented in Table 1.

Figure 1

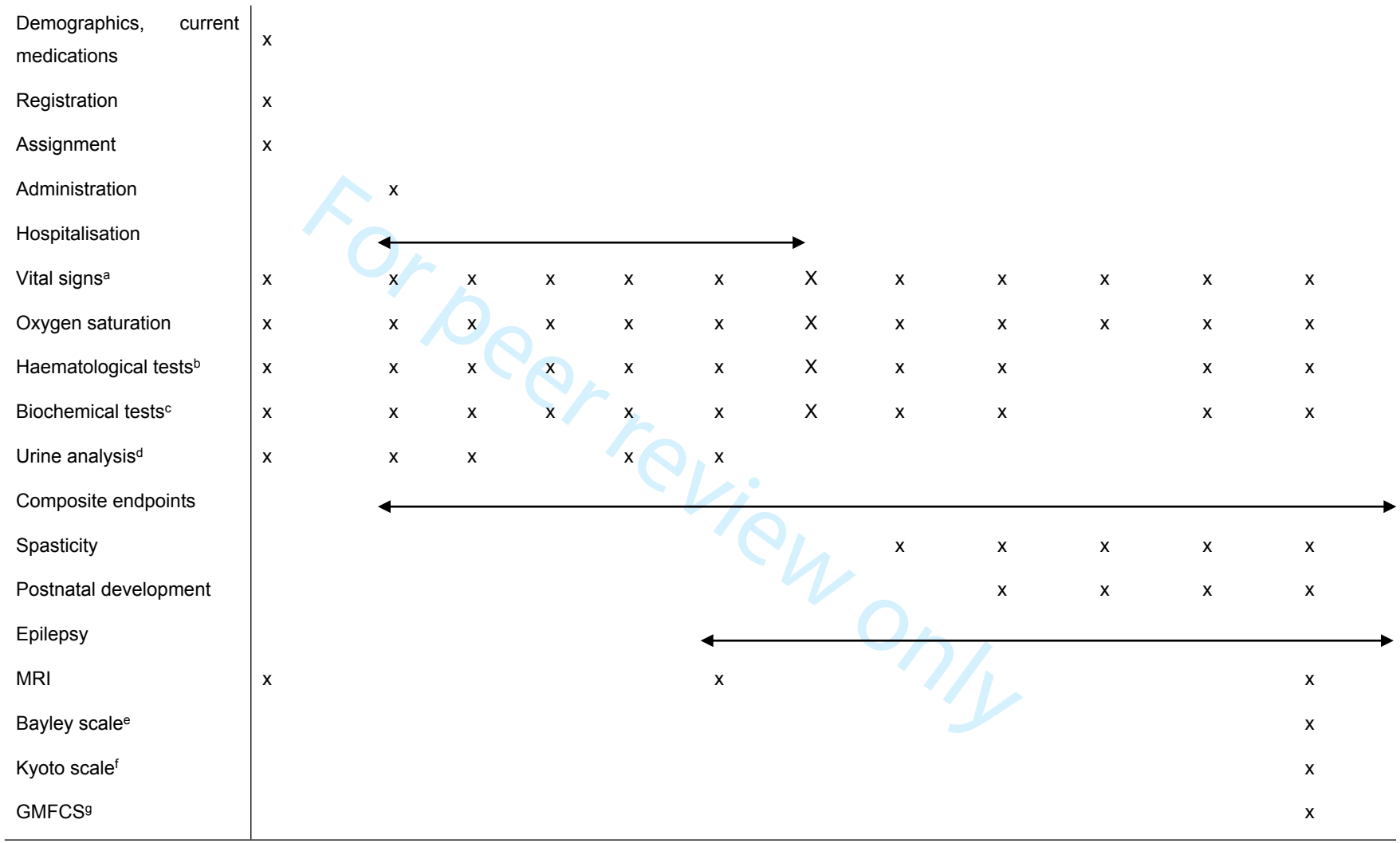
Treatments and assessments	Registration	Day 0	Day 1	Day 3	Week 1	Week 2	Week 4	Week 12	Week 26	Week 38	Week 52	Week 78
Agreement	x											

Study framework

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

Table 1

Schedule of interventions and assessments



[a]Blood pressure, pulse rate, and body temperature

[b]Red blood cell count, haemoglobin, haematocrit, white blood cell count, white blood cell fraction (basophils, eosinophils, neutrophils, lymphocytes, monocytes),

and platelet count

[c]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.

[d]pH, urine protein, urine occult blood, and urine sugar

[e]Bayley Scales of Infant and Toddler Development Third edition

[f]Kyoto Scale of Psychological Development 2001

[g]Expanded and Revised Gross Motor Function Classification System

Study endpoints

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

1) Incidence of composite endpoints (death, continuous respiratory support, or continuous use of vasopressors or pulmonary vasodilators)
2) Mortality and overall survival
3) Duration of continuous respiratory support: The definition of respiratory support is the status of conducting artificial ventilation with tracheal intubation.
4) Duration of continuous use of vasopressors or pulmonary vasodilators: dopamine, dobutamine, adrenaline, noradrenaline, milrinone, vasopressin, *dl*-isoprenaline hydrochloride, *l*-isoprenaline hydrochloride, nitric oxide, epoprostenol sodium, nitroglycerin, and alprostadil alfadex
5) The Bayley Scales of Infant and Toddler Development Third Edition [28] score at 78 weeks
6) Developmental quotient as per the Kyoto Scale of Psychological Development 2001 [29] at 78 weeks
7) Assessment of postnatal development such as head control, rolling, sitting, crawling, unaided walking, and saying several meaningful words

8) Presence of spasticity: The definition of spasticity is the status of increased muscle tone or increased deep tendon reflex.

9) Presence of epilepsy: The definition of epilepsy is based on the International League Against Epilepsy.[30]

10) Magnetic resonance imaging score: The scoring system is based on the report of Barkovich et al.[31]

11) The score of Expanded and Revised Gross Motor Function Classification System [32] at 78 weeks

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

Sample size calculation

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

Statistical analysis

All analyses are based on an intention-to-treat principle. We will summarise the demographic data using descriptive statistics. The main purpose of this exploratory clinical trial is “to confirm the safety and tolerability” of the Muse cell product. Therefore, we will analyse adverse events on the safety analysis set defined as all subjects enrolled in this study and received the investigational cell product. All adverse events will be confirmed for the primary endpoint, and the proportions of the adverse events and their 95% CI based on the Clopper-Pearson method will be calculated. Overall survival, defined as the time from birth to the date of death due to any cause, will be summarised using the Kaplan-Meier method. Descriptive statistics for continuous variables and frequency and proportion for categorical variables will be calculated for each secondary endpoint. Depending on the endpoint (e.g. the duration of continuous respiratory support, continuous use of vasopressors, or pulmonary vasodilators), it will be summarised excluding patients who had been using these therapies prior to the cells administration as necessary. Statistical analysis will be performed using the SAS software (SAS Institute, version 9.4, North Carolina, USA). Statistical

significance will be defined as p <0.05. Some endpoints, including the provision of respiratory support and the use of vasoactive drugs, may be affected by pre-enrolment condition, the effects of these potential baseline differences will not be adjusted in the analysis.

Monitoring and auditing

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

Status of this trial

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

University Hospital from February 2020 to July 2021, and the study will be terminated in September 2023.

ETHICS AND DISSEMINATION

Ethical approval

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

Patient consent for participation

The investigators and trained clinical research coordinators will introduce the trial to patients' legal representatives with prepared information sheets and informed consent forms (Supplementary file). The investigator will obtain written consent to participate in the trial. Subjects will be identified during the data collection using a subject identification code. All personnel involved in this study will take the best

possible precautions to ensure the protection of patients' personal information.

Dissemination

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

DISCUSSION

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

Perinatal brain insult induced by hypoxia is a leading cause of cerebral palsy. Several randomised controlled trials of hypothermia therapy for HIE have been conducted,[33-38] and hypothermia is currently the sole neuroprotective therapy. However, its effectiveness is insufficient, and a novel therapy is required.

Regenerative therapy is the focus of next-generation therapy. Clinical studies with autologous UCBCs for HIE had been conducted before the development of CL2020.[10, 11] This UCBCs therapy requires additional equipment and human resources for its preparation because the newborns' umbilical cord blood has to be collected at birth, and the patients receive the first dose of prepared UCBCs within 24 hours after birth. In contrast, in our non-clinical study, single intravenous administration of Muse cells to HIE model rats 3 days after hypoxic-ischaemic injury ameliorated behavioural abnormalities up to 5 months.[22] In a non-clinical study using CL2020, the treatment effect was exerted at even 7 days after insult by hypoxic ischaemia. In addition, a single dose of CL2020 administered via the vein at the subacute (about 9 days after onset) and chronic phases (about 30 days) was effective in a mouse lacunar stroke model.[26] Thus, we set the administration of Muse cells to human neonates between 5 and 14 days after birth, which means that physicians and patients' families can afford the time to decide or prepare the treatment based on the patient's condition or seek other opinions.

We held a consultation meeting about the main clinical trial design, including the timing of administration as above with the Japanese regulatory authority,

Pharmaceutical and Medical Devices Agency, and they agreed to our proposed design for this trial. We will perform a randomised placebo-controlled clinical trial to evaluate the effectiveness of CL2020 for HIE after confirming the safety and tolerability of its intravenous administration in neonates.

Herein, we present the overall design of this single-centre, open-label, dose-escalation clinical trial of Muse cell products in HIE patients with hypothermia. This clinical trial is the first clinical application of CL2020 in neonates based on our non-clinical study results. If we can verify that this product is safe and well-tolerable in neonates, its application may expand to other disorders in neonates and children.

Funding

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Contributorship Statement

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

Competing Interests

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

Acknowledgements

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

Data availability statement

The datasets generated and analysed during this study will not be publicly

available due to the confidentiality clause. This clinical trial is the first study for neonates in Japan to investigate the safety and dosage of Muse cell-product in a small group for ischemic hypoxic encephalopathy conducted in single centre. Data on individual subjects obtained in this clinical trial will not be disclosed at this time for the protection of personal information, because the risk of “re-identification” is high due to small number of enrolled patients.

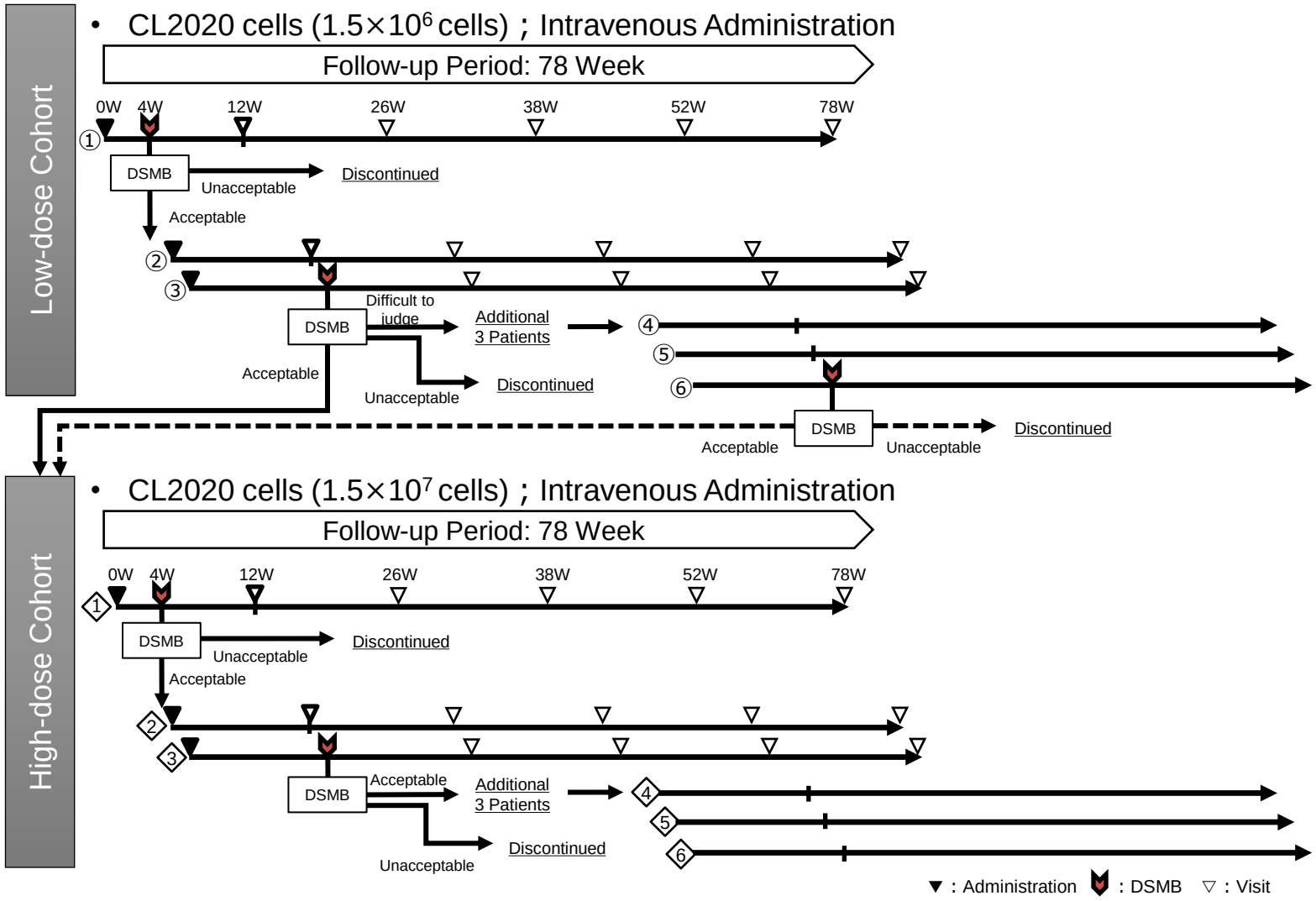

Figure 1 Study framework

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代諾者用

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 の安全性及び忍容性を検討する用量漸増臨床試験」～

現在、私たち（担当医師）は、患者さんの協力を得て、開発中の再生医療等製品である CL2020 の安全性と効き目を調べるための臨床試験（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です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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目次

１．治験について

新しい薬や製品（再生医療等製品[注]：組織・細胞加工製品または遺伝子治療製品）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。

[注] 再生医療等製品：以下に当てはまる製品のことで、政令（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（医師主導治験）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」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[注]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になります。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。

[注] 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。

＜新しい「製品」の開発の仕組み＞

基礎試験	動物や病気のモデルを使って、「製品の候補」の性能、治療効果と安全性を評価します。
▼	
治 験 **[臨床試験]**	患者さんにご協力いただき、「治験製品」の性能、治療効果と安全性を評価します。 ＜今回の治験はこの初期段階にあたります。＞
▼	
厚生労働省へ申請 ▶ **審議･承認**	
▼	
発　売	みなさんのご協力により、１つの新しい「製品」が誕生します。
▼	
製造販売後調査	「製品」として実際に発売されてからも、「製品」の性能、治療効果と安全性について調査を行い、標準的な治療法を検討します。

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
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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/)

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＜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、製薬会社が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

低酸素性虚血性脳症（ていさんそせいきょけつせいのうしょう）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、運動麻痺（うんどうまひ）、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）、精神発達遅滞（せいしんはったつちたい）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 時間継続する低体温療法（ていたいおんりょうほう）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で脳の負担を減らし、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 [redacted] SSEA-3陽性間葉系幹細胞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」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（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 [redacted] 細胞（ヒト [redacted] 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。[redacted] 心筋梗塞（検証的試験※2）、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（探索的試験※3）、脳梗塞、及び表皮水疱症（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（いろう）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、医師が不適当と判断した場合

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[redacted]で希釈し、そのうちの[redacted]（約150万個の細胞）を[redacted]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[redacted]で希釈し、その[redacted]（約 1,500 万個の細胞）を[redacted]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。

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＜治験スケジュール＞

規定日		同意	本登録	投与	1 日後	3 日後	1 週後	2 週後	4 週後	12 週後	26 週後	38 週後	52 週後	78 週後	中止時	追跡調査（治験全体の終了まで）
生後日齢		3～14	3～14	5～14	6～15	8～17	12～21	19～28	33～42	89～98	187～196	271～280	369～378	551～560		
Day（投与日=Day0 を基準）		～0	～0	0	1	3	7	14	28	84	182	266	364	546		
検査・観察日の許容範囲				0	0	±1	±2	±4	±7	±14	±14	±28	±28	±28	±28	
同意		●														
登録			●													
治験製品投与				⇩												
原疾患・合併症・母体背景の確認			●													
身長・体重・頭囲			●						●	●	●	●	●	●		
バイタルサイン（血圧、脈拍（心拍）数、体温）、酸素飽和度			●	●	●	●	●	●	●	●	●	●	●	●	●	
臨床検査★1	血液学的、血液生化学		●	●	●	●	●	●	●	●	●		●	●	●	
	血液ガス、凝固		●	●	●	●	●									
	感染症		●													
	随時尿		●	●	●		●	●								
痙性（けいせい）の確認 ★2										●	●	●	●	●		
頸定（けいてい）、寝返り、座位、はいはいの確認 ★3										●		●	●	●		
独歩（どっぽ）、有意語（ゆういご）の確認 ★4													●	●		
てんかんの確認								←―	―	―	―	―	―	―→		
対面式乳児発達検査 ★5														●		
頭部 MRI ★6			●					●						●	●	
GMFCS-E&R ★7														●		
併用薬・併用療法の確認			←―	―	―	―	―	―	―	―	―	―	―	―→	●	
状況確認																●

★の項目については次ページで説明します。

★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	**血液学的検査** 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 **血液生化学的検査** AST、ALT、ALP、γ-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 **血液ガス** pH、二酸化炭素分圧（pCO_2）、過剰塩基（BE） **凝固検査** PT、APTT、PT-INR、フィブリノーゲン、アンチトロンビンⅢ **感染症検査** HB_S 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えること）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が、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もあります。

★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も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

さんに直接的な利益が得られるかどうかは不明ですが、ご参加いただくことで、新生児低酸素性虚血性脳症の今後の新たな治療法へとつながる可能性があります。なお、「4.「CL2020」について」の項で記載されている動物試験で認められたように、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。ただし、今回の CL2020 の細胞を投与しても、十分な改善が認められず、低酸素性虚血性脳症による後遺障害を残してしまう可能性もあります。

予測される不利益について

治験製品を使用したときに起こった、あらゆる好ましくない症状や病気の徴候（ちょうこう）、臨床検査値の変化を「有害事象（ゆうがいじしょう）」といい、治験製品との関連性は問いません。そのため、「治験製品が原因である」、「治験製品が原因と疑われる」もの以外に「治験製品とは関連がない」ものが含まれます。有害事象の中で「治験製品が原因である」もしくは「治験製品が原因と疑われる」と判断された事象を「副作用（ふくさよう）」といいます。

＜CL2020 の細胞の投与で予測される主な副作用＞

- **感染症**

CL2020 は健康であることが確認された成人ドナー（提供者）の骨髄液を原料として製造しています。そのため、ドナーに由来するウイルス等に感染する可能性を完全に否定することはできません。しかし、[redacted]各種のウイルス検査を行って、ウイルスが検出された成人ドナーを除外しています。更に最終製剤において、各種ウイルスが検出されないことを確認して出荷されています。

その他製造に用いる生物に由来する原料および材料は、医薬品等の品質、有効性ならびに安全性を確保するために定められた国の基準（生物由来原料基準）に基づいたものを用いているため、ウイルスや病原体等の伝播（でんぱ）のリスクは低いと考えられます。しかしながら、[redacted]ウイルスや病原体等の伝播のリスクは完全には否定できません。

- **原料および材料によるアレルギー**

本治験製品を投与した際、製造に使用される[redacted]に対するアレルギー

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

減少し、そこで赤血球が破壊されて貧血になる状態）（10.3%）、播種性血管内凝固（体の血管の中で血栓（血の塊）ができやすくなったり、容易に出血したりする状態）（2.6%）、静脈閉塞性肝疾患（何らかの原因で肝臓の微小静脈が閉塞することにより壊死が起こり、肝障害を起こした状態）（2.6%）

④ 免疫応答

発疹（2.6%）、口内炎（2.6%）、顔面腫脹（2.6%）、アレルギー性輸血反応（2.6%）、眼瞼浮腫（2.6%）、皮膚剥脱（2.6%）

＜CL2020 の細胞の投与でヒト・動物に認められた事象＞

- **ヒトに対する臨床試験（治験）**

CL2020 は、開発中の製品であり、現在、海外および日本でもまだ販売が承認されていません。[redacted]心筋梗塞（検証的試験）、脊髄損傷、ALS 及び 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（探索的試験）、脳梗塞、表皮水疱症の患者さんを対象とした治験が完了しています。[redacted]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。[redacted]

[redacted]

- 動物に対する試験の結果

[redacted]

[redacted]

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）した後でも、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。

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。

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られます。

ただし、下記の場合などは、補償されないことがあります。

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

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

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

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」をご覧ください。

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。

11．費用の負担について

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。

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学機構から振り込むことになります。なお、口座番号、電話番号などの個人情報は厳重に管理し、振り込みの手続きのみに適切に使用いたします。

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けることになります。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、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になります。

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

た後でも、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、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、あなたやあなたの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。

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く場合があります。

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。

14. 守っていただきたいことについて

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。

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、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、必ず私たちに連絡してください。

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は、治験に参加される前に必ず私たちに伝えてください。
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。

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場合には、いつでも連絡してください。

15. 利益相反について

利益相反（COI：Conflict of Interest）とは「主に経済的な利益関係によって公正かつ適正な判断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

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 知的財産権（ちてきざいさんけん） 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。

整理番号：312005
治験実施計画書番号：CAMCR-014
名古屋大学医学部附属病院　第 3.0 版
作成日：2021 年 11 月 9 日
代諾者用

＜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）：[redacted]　内線[redacted]

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

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）：[redacted]

＜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。

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治験実施計画書番号：CAMCR-014
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代諾者用
ＩＤ番号：　　－　　－
一枚目　カルテ用　　口(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

整理番号：312005
治験実施計画書番号：CAMCR-014
名古屋大学医学部附属病院　第 3.0 版
作成日：2021 年 11 月 9 日
代諾者用
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。

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振込先口座	銀行・信用金庫 農業協同組合												支店
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フリガナ					口座番号 ※7桁未満の場合は、右詰で先頭に0をつける								
口座名義													
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	TEL：　　－　　－												

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代諾者用

三枚目　患者さん用　口(再同意)

同意文書

名古屋大学医学部附属病院長　殿

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	TEL：　　－　　－												

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

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1,2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	n/a
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a; This is a single-arm, dose-escalation trial.
Objectives	#7	Specific objectives or hypotheses	9
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			

Study setting	#9	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
Eligibility criteria	#10	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
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12
Interventions: modifications	#11b	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
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	#12	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
Participant timeline	#13	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
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined,	17

		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	
Data collection plan: retention	#18b	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
Data management	#19	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
Statistics: outcomes	#20a	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
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a
Statistics: analysis population and missing data	#20c	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			
Data monitoring: formal committee	#21a	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

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12,16
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	19
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19, 20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	20
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	24

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	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
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a; Authorship eligibilities were confirmed by standard material.
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	24
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	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