PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Matsuyama, Nao; Shimizu, Shinobu; Ueda, Kazuto; Suzuki, Toshihiko; Suzuki, Sakiko; Miura, Ryosuke; Katayama, Akemi; Ando, Masahiko; Mizuno, Masaaki; Hirakawa, Akihiro; Hayakawa, Masahiro; Sato, Yoshiaki

VERSION 1 – REVIEW

REVIEWER	Liley, Helen The University of Queensland, Mater Research Institute
REVIEW RETURNED	29-Oct-2021

GENERAL COMMENTS	This interesting paper describes the protocol for an early-phase, dose escalation study of the use of multilineage-differentiating stress-enduring cells for the treatment of hypoxic ischaemic encephalopathy in human newborn infants. In general, the paper reports a protocol in a way that is clear, near complete and explains the clinical trial. T The primary outcome for the study is the incidence of adverse events until 12 weeks after administration of the study treatment. The paper reports that recruitment of 9 of 12 planned participants has occurred, and a follow-up phase (to 1 ½ years) is still underway. The reporting checklist based on the SPIRIT guidelines has been completed. The trial has been registered in clinical trial registries and approved by a human research ethics committee and relevant regulatory agencies. Some of the authors have noted potential conflicts of interest that relate to grant funding, consultation fees and holding of a patent related to the study treatment. A few additional details of the protocol would be needed to allow replication by another study group. Specifically: 1. The objective of the study is “to confirm the safety and tolerability” of the study treatment. The recording of adverse events appears to address safety. What measures are being used to assess tolerability? 2. No reference specifying precise details of the preparation of the cell preparation used in the protocol is mentioned in the Methods.

	Although the Introduction cites other human and animal studies using Muse cells, the specific reference given in the Methods for the product (reference 26) only states; “The clinical-grade Muse cell–based product CL2020 was produced from human MSCs by exposing the cells to the combination of stresses and were confirmed to be positive for both pluripotency marker stage-specific embryonic antigen-3 and mesenchymal marker CD105 but are negative for white blood cell marker CD45” and states that further information are available from a corresponding author “upon reasonable request”. Can more precise and comprehensive details about the preparation be provided either as a reference or in a supplement? It would seem reasonable that this material would explain the method of preparation in detail, as well as donor source, storage method and duration, and any quality-checking procedures prior to administration. 3. The only stipulation about the health of the participants at the time of recruitment appears to be the exclusion criteria on page 11, which include the non-specific exclusion #9 for “severe complications”. The short-term outcomes after rewarming of infants with initially moderate or severe HIE can be quite variable, with some still quite encephalopathic as well as manifesting residual systemic consequences well after the completion of therapeutic hypothermia, while others are asymptomatic or nearly so. Is #9 intended to exclude those with ongoing encephalopathy or other organ dysfunction, or can the reader assume that the participants had a range of ongoing health problems at the time of study treatment? 4. The Methods section states on page 10, line 157 that a maximum of 12 neonates would be recruited but page 17, line 249 states; “a scheduled number … of 12”, and the actual number enrolled to date is reported as 3 in the low dose group and 6 in the high dose group. Are another 3 participants intended to be recruited, or if not, why was recruitment terminated after 9 recruits? The termination date of the study (September 2023) suggests recruitment may be continuing up to 18 months before this, i.e., March 2022. 5. On page 10, inclusion criterion #5, is the necessity for heart rate ≥100/min and SpO2 ≥90% only at the time of screening for eligibility for study treatment (after rewarming) or is this at any time during hospital stay? If at any time, this could exclude some infants with concomitant respiratory disease, and also those who have asymptomatic bradycardia while undergoing hypothermia treatment. 6. There is some explanation in the Discussion of the biological justification for the relatively long window during which treatment can be administered (5-14 days after birth). How, within this window, was or will the decision be made for any particular time point for treatment? Could the timing affect the response (either beneficial or adverse responses to treatment)? How will this be considered in the analysis? 7. Does the prohibition of “processed cell products” (P12, line 45) include red cell transfusion for anaemia? Are the prohibitions for the entire 18 months or just during the 12 weeks until censoring of adverse event outcomes? 8. The primary endpoint is described as the incidence of adverse events until 12 weeks after administration (P16, line 217). Was there a specific list of reportable events? 9. Why was the BSID III chosen for the 78-week outcome? Will the BSID IV not be more readily available and useful at the time of outcome assessment?

	10. Some of items on the list of Study endpoints (P16 and 17) are clinical events that are might pre-date study treatment. These include the provision of respiratory support, the use of vasoactive drugs and the first of the planned magnetic resonance imaging studies. Could some of these be baseline variables as well as, or instead of study endpoints? How will this be accounted for in the analysis? 11. Is there a rationale for analysing the study results on an as-treated as well as an intention-to-treat basis? This would be common in studies examining safety. 12. How will any attrition from the study be managed in the statistical analysis? 13. What events will be considered for the Kaplan-Meier analysis? Will this include minor adverse events or is there a threshold for severity? 14. The model parent information sheet and consent form has not been provided in an appendix, as suggested by the SPIRIT checklist. In addition to the above questions about the methods, the following are minor points the authors may wish to address: 15. It would be helpful for the title to include mention that this is a cell-based therapy. CL2020 is not a term that will be familiar to most readers. 16. In the abstract line 39, the term “proper hypothermia” will not be familiar to most readers. Perhaps “a course of therapeutic hypothermia” would be better? 17. In the Introduction, it is probably a misinterpretation of the paper by Kurinczuk et al. to conclude that they reported a global incidence of HIE. The estimate is mostly calculated from studies in high income countries, and the rate in countries with lower or very inequitably distributed healthcare resources, particularly for antenatal and intrapartum care, is widely assumed to be much higher. The Kurinzuk study acknowledges this and other potential sources of bias in the estimate, including the fact that it is based on some studies from the 1990’s, but also several studies from the 1970's and 1980's. Contemporary rates may differ. The much lower rate in Japan is interesting and may, in some ways illustrate the point. 18. Page 7 line 107; there is a mismatch of a singular noun (“intravenous administration”) with a plural verb (“are expected”).

REVIEWER	Gunn, Alistair The University of Auckland, Physiology
REVIEW RETURNED	31-Oct-2021

GENERAL COMMENTS	Protocol papers should report planned or ongoing studies. Patient recruitment was performed in Nagoya University Hospital from February 2020 to July 2021, and the study will be terminated in September 2023. Thus this is an ongoing study, and so is suitable as a protocol. Nevertheless, all subjects have been recruited. This leads to a lot of confusion, or at least it confused this respondent. I wonder if the study should be reported in the past tense except for the forthcoming ND followup? Stem cell therapy is of considerable promise, but is not yet established as effective; thus there will interest in this study. Many aspects of the trial need to be clarified, or more detail given of the reasons for the decisions

	Why was only one dose given?. Multiple animal studies suggest that more than one dose is needed to achieve optimal outcomes after HI. e.g. Tayla R Penny et al Brain Res 2020;1746:147001. Why will the cells be given between 5 and 14 days of age? There is no large animal data supporting such a long delay, particularly with single dose Tx. In preterm fetal sheep, tx at 12 h after was protective, while it was not protective at 5 days. E.g. Exp Neurol. Jingang Li 2016;283(Pt A):179-87. In a similar study, using 3 doses of cells was protective when the first dose was given at 24 hours. There is no large animal evidence that I can find supporting substantially later times. The authors have rodent data for an effect of treatment at day 3. None of this supports tx after day 5. The primary outcome is adverse events, but the timing after HI is likely to affect vulnerability to adverse effects and responsiveness. Why did not you not aim for a more realistic window? Certainly for a phase II or III trial it would be vital. This should be acknowledged as a limitation of this safety study. As best I can tell, adverse events are not defined any where. What type of problem were you looking for? Is it primarily hypersensitivity reactions? If yes, please state. Whole body and head MRI at the time of ND followup would help rule out the potential for inappropriate local growths. Some forms of stem cell are known to form neuroblasts. Is this planned? If it is not planned, why not? Trivia. In many places the phrasing of the MS is difficult to follow or nonstandard. The authors should consider working with an English editor. One example of confusion: “This clinical trial was named”. Does this mean that the trial is in the past, or that it is proposed to start in the future? What is proper hypothermia therapy? Assessment of postnatal development is vague. What are you comparing development against? What does 9） Severe complications mean? Why were corticosteroids prohibited? Some NICUs use hydrocortisone therapy routinely for hypotension at doses that do not suppress immune function. Why is the dose specified as prednisolone?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

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Prof. Helen Liley, The University of Queensland Comments to the Author:

This interesting paper describes the protocol for an early-phase, dose escalation study of the use of multilineage-differentiating stress-enduring cells for the treatment of hypoxic ischaemic encephalopathy in human newborn infants.
In general, the paper reports a protocol in a way that is clear, near complete and explains the clinical trial.
The primary outcome for the study is the incidence of adverse events until 12 weeks after administration of the study treatment.
The paper reports that recruitment of 9 of 12 planned participants has occurred, and a follow-up phase (to 1 ½ years) is still underway.
The reporting checklist based on the SPIRIT guidelines has been completed. The trial has been registered in clinical trial registries and approved by a human research ethics committee and relevant regulatory agencies. Some of the authors have noted potential conflicts of interest that relate to grant funding, consultation fees and holding of a patent related to the study treatment.
A few additional details of the protocol would be needed to allow replication by another study group. Specifically:

Response:
Thank you for your valuable comment. We have responded to your comments below.

1. The objective of the study is “to confirm the safety and tolerability” of the study treatment. The recording of adverse events appears to address safety. What measures are being used to assess tolerability?

Response:
Thank you for your question. 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.

2. No reference specifying precise details of the preparation of the cell preparation used in the protocol is mentioned in the Methods. Although the Introduction cites other human and animal studies using Muse cells, the specific reference given in the Methods for the product (reference 26) only states; “The clinical-grade Muse cell–based product CL2020 was produced from human MSCs by exposing the cells to the combination of stresses and were confirmed to be positive for both pluripotency marker stage-specific embryonic antigen-3 and mesenchymal marker CD105 but are negative for white blood cell marker CD45” and states that further information are available from a corresponding author “upon reasonable request”. Can more precise and comprehensive details about the preparation be provided either as a reference or in a supplement? It would seem reasonable that this material would explain the method of preparation in detail, as well as donor source, storage method and duration, and any quality-checking procedures prior to administration.

Response:
Thank you for your suggestion. Unfortunately, we cannot provide the comprehensive details of manufacturing process or acceptance criteria of this product for confidentiality in the development company. However, according to your suggestion, we added the characteristics of this product as below:

Page 12, line 195; in “Intervention and follow-up” section
The clinical-grade Muse cell-based product, CL2020 (1.5 × 107 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.

3. The only stipulation about the health of the participants at the time of recruitment appears to be the exclusion criteria on page 11, which include the non-specific exclusion #9 for "severe complications". The short-term outcomes after rewarming of infants with initially moderate or severe HIE can be quite variable, with some still quite encephalopathic as well as manifesting residual systemic consequences well after the completion of therapeutic hypothermia, while others are asymptomatic or nearly so. Is #9 intended to exclude those with ongoing encephalopathy or other organ dysfunction, or can the reader assume that the participants had a range of ongoing health problems at the time of study treatment?

Response:
The reference standard of severe complication is CTCAE grade 4. The exclusion criterion #9 is also intended to exclude subjects who have problems unrelated to HIE and considered to be intolerable to the cell product at a time of enrolment. We revised the exclusion criterion #9 to "Severe complications not related to HIE" (Page 12, line 189)

4. The Methods section states on page 10, line 157 that a maximum of 12 neonates would be recruited but page 17, line 249 states; "a scheduled number … of 12", and the actual number enrolled to date is reported as 3 in the low dose group and 6 in the high dose group. Are another 3 participants intended to be recruited, or if not, why was recruitment terminated after 9 recruits? The termination date of the study (September 2023) suggests recruitment may be continuing up to 18 months before this, i.e., March 2022.

Response:
This clinical study is a traditional 3+3 dose escalation design as shown in Figure 1. There was no serious adverse event related to Muse cell product in the 3 subjects enrolled in low dose cohort. Therefore, the data safety monitoring board (DSMB) decided to move on to the high dose cohort. So, we did not enrol another 3 participants in this clinical trial.

5. On page 10, inclusion criterion #5, is the necessity for heart rate ≥100/min and SpO2 ≥90% only at the time of screening for eligibility for study treatment (after rewarming) or is this at any time during hospital stay? If at any time, this could exclude some infants with concomitant respiratory disease, and also those who have asymptomatic bradycardia while undergoing hypothermia treatment.

Response:
Informed consent is obtained after hypothermia treatment. Inclusion criteria #5 is confirmed after obtaining informed consent, and before registration of this clinical study during hospitalization. Therefore, this makes it possible to enrol infants who are stable for the condition after hypothermia therapy.

6. There is some explanation in the Discussion of the biological justification for the relatively long window during which treatment can be administered (5-14 days after birth). How, within this window, was or will the decision be made for any particular time point for treatment? Could the timing affect the response (either beneficial or adverse responses to treatment)? How will this be considered in the analysis?

Response:
We decided to administer as soon as possible within this window (5–14 days after birth) in principle after registration.
We do not intend to analyse the relationship with administration timing, because the main purpose of this clinical trial is “to confirm the safety and tolerability” of the CL2020 treatment.
7. Does the prohibition of “processed cell products” (P12, line 45) include red cell transfusion for anaemia? Are the prohibitions for the entire 18 months or just during the 12 weeks until censoring of adverse event outcomes?

Response:
Red cell transplantation is not prohibited in this clinical study. We have revised the phrase to “processed cell products except for the red blood cells”. (Page 13, line 208)

8. The primary endpoint is described as the incidence of adverse events until 12 weeks after administration (P16, line 217). Was there a specific list of reportable events?

Response:
Several adverse events were identified in this clinical trial. However, there was no serious adverse event related to the investigational product in the 9 subjects enrolled.
The clinical trial data until 12 weeks after administration are being checked and analysed. We would like to summarise the trial results and submit as another report in near future.

9. Why was the BSID III chosen for the 78-week outcome? Will the BSID IV not be more readily available and useful at the time of outcome assessment?

Response:
As you pointed out, the BSID IV was available at the initiation of this clinical trial. However, it has not been validated for Japanese children. This clinical trial is located only in Japan. Therefore, we chose the BSID III in this trial.

10. Some of items on the list of Study endpoints (P16 and 17) are clinical events that are might pre-date study treatment. These include the provision of respiratory support, the use of vasoactive drugs and the first of the planned magnetic resonance imaging studies. Could some of these be baseline variables as well as, or instead of study endpoints? How will this be accounted for in the analysis?

Response:
As you mentioned, the provision of respiratory support and the use of vasoactive drugs might occur before enrolment in this study. The main purpose of this exploratory clinical trial is “to confirm the safety and tolerability” of the Muse cell product. Therefore, we will summarise these demographic data using descriptive statistics, and we will not adjust the effects of these potential baseline differences (e.g. the provision of respiratory support, the use of vasoactive drugs and the first of the planned MRI etc.) in the analysis.

11. Is there a rationale for analysing the study results on an as-treated as well as an intention-to-treat basis? This would be common in studies examining safety.

Response:
We will analyse adverse events on the safety analysis set defined as all subjects who are enrolled in

this study and received the investigational cell product.

Page 18, line 258; in “Statistical analysis” section
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.

12. How will any attrition from the study be managed in the statistical analysis?

Response:
All subjects including patients who die or drop out of the observation period in this study will be analysed. However, depending on the endpoint (e.g. continuous respiratory support, continuous use of vasopressors, or pulmonary vasodilators), it will be summarised excluding these patients as necessary.

13. What events will be considered for the Kaplan-Meier analysis? Will this include minor adverse events or is there a threshold for severity?

Response:
Overall survival will be analysed using the Kaplan-Meier analysis. We explain this in the “Statistical analysis” section in the manuscript as below:
Page 18, line 258; in “Statistical analysis” section
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 $p < 0.05$.

14. The model parent information sheet and consent form has not been provided in an appendix, as suggested by the SPIRIT checklist.

Response:
We did not attach the model parent information sheet and consent form, because this clinical trial is implemented only in Japan and we prepared the informed consent form only in Japanese.

In addition to the above questions about the methods, the following are minor points the authors may wish to address:

15. It would be helpful for the title to include mention that this is a cell-based therapy. CL2020 is not a term that will be familiar to most readers.

Response:
Thank you very much. We have changed the title in accordance with your suggestion as follows.

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

16. In the abstract line 39, the term “proper hypothermia” will not be familiar to most readers. Perhaps “a course of therapeutic hypothermia” would be better?

Response:
Thank you for your suggestion. We have fixed it as below:

Methods and analysis in “Abstract” section
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.

17. In the Introduction, it is probably a misinterpretation of the paper by Kurinczuk et al. to conclude that they reported a global incidence of HIE. The estimate is mostly calculated from studies in high income countries, and the rate in countries with lower or very inequitably distributed healthcare resources, particularly for antenatal and intrapartum care, is widely assumed to be much higher. The Kurinzuk study acknowledges this and other potential sources of bias in the estimate, including the fact that it is based on some studies from the 1990’s, but also several studies from the 1970’s and 1980’s. Contemporary rates may differ. The much lower rate in Japan is interesting and may, in some ways illustrate the point.

Response:
Thank you for your proper advice. We have revised the manuscript based on report by Kurinczuk et al.

Page 5, line 76; in “Introduction” section
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 and 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]

18. Page 7 line 107; there is a mismatch of a singular noun (“intravenous administration”) with a plural verb (“are expected”).

Response:
Thank you very much. We have fixed it as below:

Page 7, line 113; in “Introduction” section
Based on these characteristics, intravenous administration of allogenic Muse cells is expected to be an effective regenerative therapy for HIE.

Reviewer 2:
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Dr. Alistair Gunn, The University of Auckland Comments to the Author:

Protocol papers should report planned or ongoing studies.
Patient recruitment was performed in Nagoya University Hospital from February 2020 to July 2021, and the study will be terminated in September 2023.
Thus this is an ongoing study, and so is suitable as a protocol. Nevertheless, all subjects have been recruited. This leads to a lot of confusion, or at least it confused this respondent. I wonder if the study should be reported in the past tense except for the forthcoming ND followup?

Response:
We sincerely apologize for having caused any confusion. All subject had been already registered. However, this study is ongoing. Therefore, we used the present tense in “Methods and analysis” section of this manuscript.

Stem cell therapy is of considerable promise, but is not yet established as effective; thus there will interest in this study.
Many aspects of the trial need to be clarified, or more detail given of the reasons for the decisions
Why was only one dose given?. Multiple animal studies suggest that more than one dose is needed to achieve optimal outcomes after HI. e.g. Tayla R Penny et al Brain Res 2020;1746:147001.
Why will the cells be given between 5 and 14 days of age? There is no large animal data supporting such a long delay, particularly with single dose Tx. In preterm fetal sheep, tx at 12 h after was protective, while it was not protective at 5 days. E.g. Exp Neurol. Jingang Li 2016;283(Pt A):179-87. In a similar study, using 3 doses of cells was protective when the first dose was given at 24 hours. There is no large animal evidence that I can find supporting substantially later times. The authors have rodent data for an effect of treatment at day 3. None of this supports tx after day 5.

Response:
Thank you for your insightful comment. As we mentioned in the manuscript, we confirmed that the human allogenic Muse cells-based product, CL2020, exerted a treatment effect in the HIE rat models. In this non-clinical study, incidentally, a single dose of CL2020 was administered intravenously at 3 or 7 day after insult by hypoxic ischaemia (in preparation for submission). 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 (Stroke 2020;51:601–11). Therefore, we thought a single dose of CL2020 is effective at even a later time point like between 5 and 14 days of age.
We added these supplementary explanations in the revised manuscript as shown below:

Page 22, line 324; in “Introduction” section
“Discussion” section
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.

The primary outcome is adverse events, but the timing after HI is likely to affect vulnerability to adverse effects and responsiveness. Why did not you not aim for a more realistic window? Certainly for a phase II or III trial it would be vital. This should be acknowledged as a limitation of this safety study.
As best I can tell, adverse events are not defined any where. What type of problem were you looking for? Is it primarily hypersensitivity reactions? If yes, please state.

Response:
Thank you very much for your comment. We agree with your opinion. The timing after HI may not be ideal for the safety study. However, we considered that we should confirm the safety and tolerability of administering Muse cell product even during such a period that could be vulnerable to side effects. Adverse event is defined as a “Any untoward medical occurrence in a patient or clinical investigation subject to whom the cell product is administered and which does not necessarily have a causal relationship with this treatment” based on the ICH E2 guideline. Especially, what we are concerned about is a serious adverse event related to the investigational product administration. As this is the first clinical trial to administer CL2020 to infants, we will evaluate by confirming all adverse events.

Whole body and head MRI at the time of ND followup would help rule out the potential for inappropriate local growths. Some forms of stem cell are known to form neuroblasts. Is this planned? If it is not planned, why not?

Response:
The evaluation by head MRI was set in this clinical study but not whole-body MRI. If any abnormal symptoms develop in infants, we will check it out in regular medical examinations in additional evaluation by whole-body MRI as needed.

Trivia.
In many places the phrasing of the MS is difficult to follow or nonstandard. The authors should consider working with an English editor.
Response:
Sorry for the inconvenience. Although we had asked a company for English editing of the original version of our manuscript, we have also requested a proofreader to check our revised manuscript. We attached editing certificate by a proofreader in end of this letter.

One example of confusion: “This clinical trial was named”. Does this mean that the trial is in the past, or that it is proposed to start in the future?

Response:
Sorry for the confusion. This clinical trial is ongoing.

What is proper hypothermia therapy?

Response:
Thank you for your suggestion. We have fixed it as below:

Methods and analysis in “Abstract” section
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.

Assessment of postnatal development is vague. What are you comparing development against?

Response:
As shown in the manuscript, postnatal development is confirmed if subjects can exhibit head control, rolling, sitting, crawling, walking unaided, and saying several meaningful words, which are typical developmental milestones. In addition, we will evaluate development using Bayley Scales of Infant Development III and the developmental quotient in Kyoto Scale of Psychological Development 2001, which is the most used developmental assessment in Japan. In both evaluations, reference values had been made with many volunteers.

What does 9） Severe complications mean?

Response:
The reference standard for severe complications is CTCAE grade 4. We would exclude a subject from this study if comprehensive examination reveals that they could have health problems unrelated to HIE, and intolerable of the cell product at a time of enrolment. We revised the exclusion criterion #9 to “Severe complications unrelated to HIE”
Why were corticosteroids prohibited? Some NICUs use hydrocortisone therapy routinely for hypotension at doses that do not suppress immune function. Why is the dose specified as prednisolone?

Response:
As it is known that corticosteroids affect cell proliferation mediated by RNA transcription (Clin Dermatol. 1989;7(3):80-97), we thought that they could affect infant growth or the function of the administered cells. Therefore, we decided to prohibit high-dose corticosteroids. In hydrocortisone therapy, which is used in many NICUs for hypotension, a much smaller amount of steroids is used than is prohibited in this clinical trial. Therefore, even though we prohibit high-dose corticosteroids, the number of infants who will be excluded by this criterion is expected to be extremely limited. Conversion to prednisolone dose is used in clinical trial due to various corticosteroids existed.

Again, thank you for giving us the opportunity to improve our manuscript with your valuable comments and queries. We have worked hard to incorporate your feedback and hope that these revisions persuade you to accept our submission.