EDEN

Erythropoietin and Darbepoetin in Neonatal Encephalopathy study

Version 3
07/05/2020

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STUDY COORDINATION CENTRE: Centre for Perinatal Neuroscience. Imperial College London.

Protocol authorised by:

Name & Role	Date	Signature
Dr Sudhin Thayyil (Chief Investigator)	07/05/2020	

Chief Investigator:
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Study Coordination Centre

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Clinical Queries

Clinical queries should be directed to Dr. Sudhin Thayyil who will direct the query to the appropriate person

Sponsor

Imperial College London/Imperial College Healthcare NHS Trust (delete as applicable) is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
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http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice

Funder: National Institute for health Research (NIHR).

This protocol describes the EDEN study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

EPO	Erythropoietin
DARBE	Darbepoetin alfa
NAA	N-acetylaspartate
MR	Magnetic resonance
HIE	Hypoxic Ischemic Encephalopathy
NE	Neonatal encephalopathy
MRS	Magnetic resonance spectroscopy
LAC	Lactate
U	Units
KG	Kilograms
LMIC	Low and middle-income countries
µG	Micrograms
IV	Intravenous
SNR	Signal to Noise Ratios
EEG	Electroencephalography

STUDY SUMMARY

TITLE Erythropoietin and Darbepoetin in Neonatal Encephalopathy (EDEN) study

DESIGN 3 arm open label pilot randomised controlled trial.

AIMS To examine the physiological effects of erythropoietin (Epo) and Darbepoetin alfa (Darbe) therapy on proton magnetic resonance spectroscopy thalamic N-acetylaspartate (NAA) level in babies with neonatal encephalopathy undergoing cooling therapy.

OUTCOME MEASURES

Phase 1

Primary outcome

Optimization of the signal to noise ratios (SNR) for MR spectroscopy at 1.5Tesla and 3Tesla, using clinical acceptable scan duration at 1.5 Tesla.

Phase 2

Primary outcome

Mean (SD) of thalamic NAA level in babies treated with Epo and Darbe when compared with untreated infants for predicting adverse neurodevelopment.

Secondary outcome

Number of babies in whom thalamic NAA level could be accurately quantified in 3Telsa and 1.5Tesla MR scanners.

POPULATION 20 Adult Healthy Volunteers

220 newborns with hypoxic ischemic encephalopathy.

ELIGIBILITY

Phase 1: Adults

Inclusion Criteria

1. >18 years old.

Exclusion Criteria

1. Adults with disease.
2. Adults with metal implant fitted, such as a pacemaker or artificial joint
3. Pregnancy.

Phase 2: Infants

Inclusion Criteria

1. Age $\leq$ 24 hours
2. Birth-weight >1.8 kg
3. Gestation $\geq$36 weeks
4. Need for continued resuscitation at 10 minutes after birth and/or 10 minutes Apgar score <6
5. Cooling therapy initiated for neonatal encephalopathy within 6 hours of age as a part of standard clinical care, with an intention of continuing for 72 hours.

Exclusion Criteria

1. Major life-threatening congenital malformation.
2. Concomitant participation in other research projects

DURATION 3-year recruitment followed by a neurodevelopmental assessment between 18 and 24 months after birth.

1. INTRODUCTION

1.1 BACKGROUND

Neonatal Encephalopathy and therapeutic hypothermia

Birth asphyxia related brain injury (hypoxic ischemic encephalopathy; HIE) occurs in 2.6 (95% CI 2.5 to 2.8) per 1000 live births in the UK, and is the most common cause of death and neurodisability in term babies [1,2]. The economic burden to the treasury on support costs of neuro-disability from HIE is massive (£4 billion per year). In addition, birth asphyxia related (obstetric) claims accounted for almost half of the NHS litigation expenses in 2016/17 (approx. £2 billion), increasing by 15% from the previous year. It has been reported that the NHS cost to meet the complex life-long care needs of babies born with brain damage could be soon over £20m per child, and this situation is unsustainable to the NHS.https://news.sky.com/story/record-rise-in-babies-born-with-brain- damage-sees-nhs-compensation-bill-soar-10991025. The UK Government has recently (October 2016) announced that reducing birth asphyxia related costs is a priority area for the Government. https://www.gov.uk/government/news/improving-the-safety-of-maternity-care-in-the-nhs

The only effective treatment for HIE is whole body cooling, with an estimated saving of £100 million per annum[3] to the UK economy, since its introduction as a standard therapy in the NHS in 2007. Cooling therapy has substantially improved the outcomes of babies with HIE in the past decade. However, unacceptably high rate of adverse outcomes are still seen in cooled babies with moderate or severe HIE: death 28% (range 24– 38); cognitive impairment 24% (range 21–25); cerebral palsy 22% (range 13–28); epilepsy 19% (range 15–24); cortical visual impairment 6% (range 1–10), with combined death or moderate/severe disability 48% (range 44– 53)[4], and hence better treatments and further optimisation of cooling therapy is required.

A key roadblock in clinical translation of over 15 highly effective neuroprotective treatments in animal models[5] is the long delay between the intervention and outcome assessments in HIE. i.e. the earliest age at which neurodevelopmental outcome can be accurately assessed is 18 months. Hence, despite having over 2 dozen highly effective treatments in animal models, no further neuroprotective drugs in HIE have been introduced into the NHS in the past 10 years.

Magnetic resonance biomarkers to accelerate clinical translation

We undertook a meta-analysis of all cerebral MR biomarkers for prediction of long term adverse neurodevelopmental outcome following NE [7]; the most robust predictor was derived from basal ganglia or thalamic proton MR spectroscopy (MRS), and is the ratio of the metabolites lactate and N-acetyl aspartate (Lac/NAA).

However, these data were based on small single-centre studies, in encephalopathic infants before the introduction of therapeutic hypothermia into routine clinical practice. Furthermore, there were limited data on absolute quantification of metabolite concentrations, which offer more specific surrogate outcome measures ideally suited to multi-centre trials.

We subsequently developed cross platform 3Tesla MR spectroscopy sequences and then conducted a large prospective study to examine the prognostic accuracy of MR biomarkers in a multi- country setting (8 tertiary hospitals in the UK and USA) involving all common scanner makes (Siemens, Philips and GE). These data showed that thalamic N-acetyl aspartate levels (NAA) at 1 week predicted neurodevelopmental outcomes at 2 years with an area under curve of 0.99[8]. An accompanying commentary in the Lancet Neurology (Nov 2018) by leading academics explains

how this biomarker as a surrogate outcome measure in next generation clinical trials can accelerate clinical translation of new neuroprotective drug therapies[9].

While MR spectroscopy at 3Telsa is ideal for neonatal brain imaging, only few centres in the UK have 3T MR scanners. Many NHS hospitals continue to use 1.5 Tesla scanners and are not able to participate in neonatal neuroprotection trials. *Hence, it essential to develop comparable MR spectroscopy sequences at 1.5 Tesla for conducting multicentre studies in the UK.*

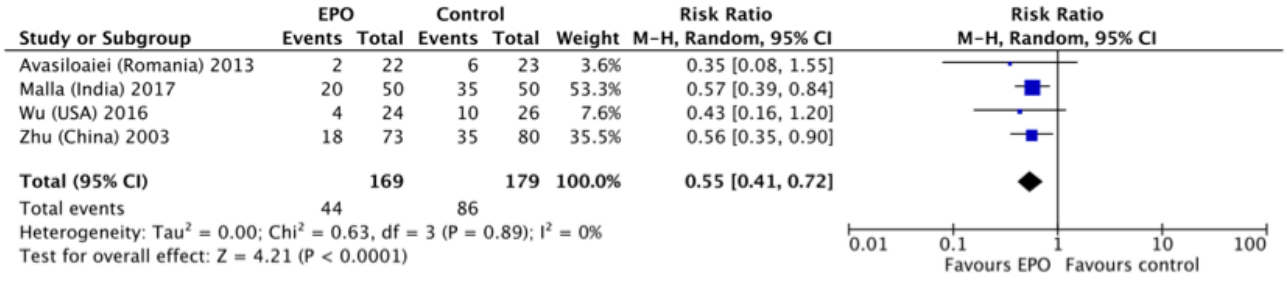
Erythropoietin therapy in neonatal encephalopathy

Erythropoietin (Epo) is a widely used and FDA approved drug for treating anaemia in various age groups, including newborn infants. Over 34 randomised controlled trials recruiting over 3500 premature babies have reported the safety and efficacy of Epo therapy for anaemia of prematurity.

Several recent reviews have highlighted Erythropoietin as one of the most promising therapies to augment hypothermic neuroprotection. Epo has both acute effects (anti-inflammatory, anti-excitotoxic, anti-oxidant, and anti-apoptotic) and regenerative effects (neurogenesis, angiogenesis, and oligodendrogenesis) essential for the repair of injury and normal neurodevelopment in animal models[5]. Of the long list of highly effective drugs in animal models of HIE and early clinical studies, Epo is the most promising. It is the only drug with a long therapeutic window (at least 24 hours), is widely available, inexpensive, and can be easily administered on a once a day dosing schedule. It has been extensive evaluated in large randomised controlled trials for anaemia of prematurity and has a proven safety profile in newborn infants[10].

Furthermore, pharmacokinetics of erythropoietin in term encephalopathic babies undergoing cooling therapy is well studied. A dose of 1000 U/kg every 24 hours provides adequate levels associated with optimal neuroprotection in pre- clinical models and are very well tolerated[11].

We conducted a systematic review of all the randomised controlled trials of Epo in HIE published to date. The pooled data suggest Epo reduces death or disability at 12 months or more after NE (risk ratio 0.61 (95% CI 0.48 to 0.78). However, the individual studies were small and of poor quality, and all except one study examined the efficacy of Epo monotherapy in low and middle-income countries (LMIC), without the adjunct use of therapeutic hypothermia.



Darbepoetin

Darbepoetin, a long acting erythropoiesis stimulating agent, has dual erythropoietic and potential neuroprotective effects. It has been extensively evaluated in newborn infants for its erythropoietic effects. The DANCE study (Darbepoetin administered to neonates undergoing cooling for encephalopathy) randomised 30 term infants with moderate to severe HIE to placebo (n=10), 2 µg/kg Darbe (n=10) or 10 µg/kg Darbe (n=10) IV. At 2 and 10 µg/kg Darbe, t1/2 was 24 and 32

hours. A dose of 10 µg/kg dose achieved an AUC in the neuroprotective range, and a terminal t1/2 of 53.4 hours when compared to the 2 µg/kg dose. No side effects attributable to Darbe were reported.

Safety profile of EPO and Darbe

Erythropoietin stimulating Agents (ESA) are glycoprotein regulating red blood cell production and are used to treat anaemia due to chemotherapy, kidney disease, major surgery, or certain treatments in HIV in adult and children.

Most complications of ESAs have been derived from studies in adults. The more common complications of prolonged ESA treatment in adults and children are arthralgia, embolism and thrombosis, headaches, flu-like symptoms, hypertension, skin reactions, stroke, hypertensive crisis, respiratory tract congestion, seizure, thrombocytosis and pure red cell aplasia[15].

Several individual studies have shown a potential trend associating ESA use with increased cancer progression[16-18]. Aapro et al[19] in systematic review concludes that the balance of current evidence does not support an effect of ESAs on stimulating tumor progression in adults receiving chemotherapy and treated for a minimum of 4 weeks and up to 12 months. A Cochrane review by Tonia et al[20] showed that the available data are insufficient to evaluate the effect of ESAs on tumour response.

EPOETIN ZETA	DARBEPOETIN ALPHA
Common or very common Asthenia; dizziness **Uncommon** Intracranial haemorrhage **Rare or very rare** Angioedema **Frequency not known** Aneurysm; cerebrovascular insufficiency; hypertensive encephalopathy; myocardial infarction; myocardial ischaemia	**Common or very common** Hypersensitivity; oedema

Table 1. Specific complications from Epoetin Zeta and Darbepoetin Alpha in children. [21]

However, these adverse effects have not been reported in Epo- or Darbe-treated neonates.

In clinical trials that evaluated possible undesired side-effects from epo and darbe treatment in term infants found no difference in adverse outcomes or complications, compared to control group, as outlined in Appendix 1. Erythropoietin dosing regimens in neonates for intravenous infusion vary between 250IU/kg to 2500IU/kg, 3 to 7 doses for a maximum of two weeks (Appendix 1). In adults, Epo dosing regimens vary between 50-600units/kg 3 times weekly for a minimum of 4 weeks[15].

An EPO-induced high haematocrit can also cause brain injury[22]. However, babies with NE usually have lower haematocrits from perinatal events or from frequent blood sampling for clinical monitoring[22-23]. Zhu et al[23] compared two groups of term infants affected with NE: 83 received erythropoietin and 84 received conventional treatment. Haemoglobin and reticulocyte levels decreased significantly at 2 weeks after birth in the control group, but this decrease was prevented in the erythropoietin group.

Basegra et al[24,] in the DANCE study reported that none of the most common complication of prolonged darbe treatment were found in the darbe- treated term infants. And more recently, Juul et al[25] randomised 741 preterm infants to high dose erythropoietin versus placebo and found no meaningful differences between groups in any serious adverse events, including those known to occur in adults who receive long-term erythropoietin treatments.

Benefits of the study

The data from this study will provide key feasibility information for future neuroprotection trials. Firstly, the study will inform if multicentric trials using thalamic NAA can be conducted in a setting with both 1.5 Telsa and 3 Tesla MR scanners.
Secondly, this study will provide a Go/No Go signal for a subsequent phase II placebo controlled randomised controlled trial to examine the clinical benefits of Epo on neurodevelopmental outcomes in cooled infants with neonatal encephalopathy.

3. STUDY OBJECTIVES

Phase I: MR spectroscopy sequence development

To develop comparable 1.5 Tesla and 3Tesla MR spectroscopy sequences for obtaining similar thalamic N-acetyl aspartate levels

Objectives

1. To obtain similar signal to noise ratios (SNR) for MR spectroscopy at 1.5Tesla and 3Tesla, using clinical acceptable scan duration at 1.5 Tesla.
2. To compare the thalamic NAA levels in healthy adult volunteers scanned at 1.5 Telsa and 3Telsa MR scanners.

Phase 2: Erythropoietin and Darbepoetin therapy

To examine the physiological effects of erythropoietin or Darbepoetin therapy on proton magnetic resonance spectroscopy thalamic N-acetylaspartate (NAA) level in babies with neonatal encephalopathy undergoing cooling therapy.

Objectives

1. To examine the feasibility of obtaining thalamic NAA levels at 3Telsa and 1.5 tesla MR scanners.
2. To examine the mean values and standard deviation of thalamic NAA levels following erythropoietin or Darbepoetin therapy in babies with neonatal encephalopathy undergoing cooling therapy between 1 to 2 weeks of age.

3. STUDY DESIGN

This study will be conducted in two phases:

Phase 1: This phase will be conducted at Imperial NHS trust and Imperial College London, recruiting 20 healthy adult volunteers over a 2-month period. All volunteers will be scanned at 1.5 Tesla and 3Tesla MR scanners, and the sequences will be optimised to obtained comparable thalamic NAA levels.

Phase 2: This phase will be conducted in 15 tertiary neonatal units in the UK over a 2-year period; 7 with 3Tesla MR scanners and 8 with 1.5 Telsa MR scanner, using the optimised MR spectroscopy sequences.

Babies (>36 week gestation and birthweight >1.8kg) requiring resuscitation at birth will be screened for eligibility, and recruited if there is evidence of acute perinatal asphyxia (metabolic acidosis in cord and/or blood gas (pH<7.15; BE >-12) within 1h of birth; acute obstetric event) AND the need for continued resuscitation or ventilation at 10 minutes and a 10 min Apgar score <6

AND evidence of moderate or severe HIE on an NICHD neurological examination performed between 1 and 6h of birth AND cooling was initiated before 6 h of age. Babies with mild HIE, and those cooled after 6 hours or with life threatening congenital malformations will be excluded.

After informed parental consent, a total of 220 babies with HIE (aged <24 hours) undergoing therapeutic hypothermia will be randomised to one of the following groups

- Arm 1: Erythropoietin (1000 U/kg) IV once a day x 5 doses along with cooling therapy
- Arm 2: Darbepoetin Alpha (10 mcg/kg) IV single dose given less than 24 hours of age along with cooling therapy.
- Arm 3: Cooling only (usual care)

Electroencephalography (EEG)
Babies recruited from Imperial NHS trust will have a 16-channel video electroencephalography (EEG), and those recruited from other centres will have amplitude integrated EEG (aEEG) measurements for the first 80 hours. The video EEG will be acquired and uploaded into a cloud based sever. This system allows a variety of research EEG double reporting options. Neonatal seizures will be classified based on their diagnostic certainty into 5 levels, as defined by the Brighton Collaboration (https://www.brightoncollaboration.org/peer-review/GAIA-Neonatal-Seizures.html).

Blood samples
We will collect 0.5ml of blood as soon as possible after birth and again at 80 hours of age from all recruited babies for gene expression studies. The blood (venous or arterial) will be collected in a PAXGENE bottle, at the time of routine clinical sampling, whenever possible.

MR Imaging and spectroscopy
1.5 Telsa and 3 Telsa MR scan using the harmonised sequences will be acquired between 1 to 2 weeks after birth. Briefly this will include $T_1$and T_2-weighted imaging, and diffusion tensor imaging, a single voxel thalamic spectroscopy for absolute quantification of NAA, as reported previously (Lally et al Lancet Neurology Nov 2018). The conventional MR images will be reported using a validated scoring system, and the MR spectroscopy will be analysed using LC model and an in-house motion correction software.

3.1 STUDY OUTCOME MEASURES

Phase 1:
Primary Outcome Measures

- Similar signal to noise ratios (SNR) for MR spectroscopy at 1.5Tesla and 3Tesla, using clinical acceptable scan duration at 1.5 Tesla.

Phase 2:
Primary outcome measures

- Mean (SD) of thalamic NAA level in babies treated with Epo and Darbe when compared with untreated infants.

Secondary outcome measures

- Number of babies in whom thalamic NAA level could be accurately quantified in 3Telsa and 1.5Tesla MR scanners.

4. PARTICIPANT ENTRY

4.1 INCLUSION CRITERIA

Phase 1
Adults >18 years old.

Phase 2
All babies aged ≤ 24 hours,≥36 weeks with a birth-weight >1.8kg with clinical evidence of encephalopathy (mild, moderate or severe) within 6 hours of birth, along with evidence of an intra-partum asphyxia insult and where cooling therapy encephalopathy within 6 hours of age as a part of standard clinical care, with an intention of continuing for 72 hours.

4.2 EXCLUSION CRITERIA

Phase 1:
1. Adults with existing disease.
2. Adults with metal implant fitted, such as a pacemaker or artificial joint
3. Pregnancy

Phase 2:
1. Babies without encephalopathy.
2. Major life-threatening congenital malformation.
3. Concomitant participation in other research projects

4.3 WITHDRAWAL CRITERIA

Phase 1:
Withdrawal of consent

Phase 2:
Withdrawal of parent or physician consent

5. ADVERSE EVENTS

5.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation

but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded.

5.3.2 Serious AEs

Prespecified serious adverse events (SAEs) are:

- Systemic hypertension requiring anti-hypertensive therapy
- Polycythemia
- Disseminated intravascular coagulation (clinical bleeding/oozing requiring transfusion of blood product)
- Major venous or arterial thrombosis not related to a central line
- Pulmonary hypertension treated with inhaled nitric oxide or extracorporeal life support
- Intracranial hemorrhage visualized on head ultrasound or MRI T1 or T2 sequences
- Cardiopulmonary arrest not secondary to ETT obstruction or other mechanical issue
- Death

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to Neonatal Encephalopathy, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the West of Scotland Research Ethics Committee where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
jrco@imperial.ac.uk
CI email (and contact details below)
Fax: 0203 313 1122, attention Dr. Sudhin Thayyil
Please send SAE forms to: s.thayyil@imperial.ac.uk
Tel: 020 3313 2473 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

6.1. Neurodevelopmental evaluation

All babies will have detailed neurodevelopmental evaluation including Bayley Scales of Infant Development (BSID-III or IV if available), Gross Motor Function Classification System (GMFCS), and hearing and vision assessment between 18 to 24 months of age. Severe disability will be defined as any one of the following: both cognitive and language composite BSID-III scores <70, GMFCS level 3–5, hearing impairment requiring hearing aids, or blindness. Moderate disability will be defined as both cognitive and language composite BSID-III scores between 70 and 84 and one or more of the following: GMFCS level 2, hearing impairment with no need for amplification or a persistent seizure disorder. The outcomes will also be assessed by categorisation of these scores and by including mortality as an outcome. An adverse outcome will be defined as death or, in survivors, moderate or severe disability.

6.2. Incidental Findings

If an incidental finding is observed during a procedure which is carried out as part of the research, and it is considered a significant abnormality then the study team should report these to the PI who should take action accordingly.
Incidental finding will be feedback to the participants and to the clinical care team or participants GP in writing.

7. STATISTICS AND DATA ANALYSIS

We expect to recruit at least 60% of the eligible population and to have adequate quality data on the primary outcome for the main trial (i.e. thalamic N-acetylaspartate concentration) in >95% of the recruited babies.

We will use an unpaired t-test to estimate the difference in thalamic/basal ganglia NAA concentration between infants receiving erythropoietin and Darbepoetin alfa.

The standard deviation of [NAA] in the MARBLE study was 0.8. To detect a difference of 0.5 mmol/kg wet weight of [NAA] at 90% power and 0.017 significance, 71 infants will be required for each of the 3 groups for unadjusted analysis. The total sample size is increased to 220 to account for neonatal mortality and any unusable data due to motion artefacts. Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the West of Scotland Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

8.2.1 Phase 1:

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.2.2 Phase 2:

Informed parental consent will be obtained prior to recruitment and randomisation but since the study requires data collection as soon as possible after birth, only the blood collection may begin before informed parental consent is obtained (deferred consenting) at the same time as the clinical blood sample collection on admission.
Parents will be informed about the study at the earliest appropriate opportunity (within 12 hours of birth) and will be given the Parent Information leaflet (PIL). Parents wishing to participate in the study will be asked to sign an informed consent form and be given a copy for their records (with the PIL), their baby will be randomised into one of the three groups only after parental consent.
If the parents do not wish to participate in the study, data collection will be interrupted, and the blood samples obtained up to that point will be discarded.
Whether or not the parent(s) decide to take part in the study shall not affect the clinical decisions made during the care of the baby, nor the quality of care provided. All participants are free to withdraw from the study at any time without giving any reason and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

National Institute for Health Research and Garfield Weston Foundation, Imperial College London are funding this study.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

9. STUDY MANAGEMENT

A Trial Management Group (TMG) will be appointed and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be co-ordinated through the Study Coordination Centre. An independent Data Monitoring Committee will be appointed.

10. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed, and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy. Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.

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12. APPENDICES

APPENDIX 1: Summary of clinical studies on ESAs on term infants with NE.

Author/Paper	Population	Dose	Route	Key Results/Safety
Zhu, C.; Kang, W.; Xu, F.; Cheng, X.; Zhang, Z.; Jia, L.; Ji, L.; Guo, X.; Xiong, H.; Simbruner, G.; et al. Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. *Pediatrics* **2009**, *124*, e218–e226	167 infants (≥37 weeks' gestation), with either moderate or severe hypoxic-ischemic encephalopathy (HIE)	300 or 500 U/kg (*n* = 83) alternate days for 2 weeks or conventional (*n* = 84)	Subcutaneously (s.c.) for 1st dose; Intravenous (i.v.) thereafter	When 18-months-old, improved long-term outcomes after EPO treatment in the infants with moderate HIE, but not in those with severe HIE. Erythropoietin was well tolerated, and neither allergic reactions nor venous thromboses were observed. Liver and renal functions, as well as electrolyte levels, were not different between the erythropoietin and control groups. Haemoglobin and reticulocyte levels decreased significantly at 2 weeks after birth in the control group, but this decrease was prevented in the erythropoietin groups.
Elmahdy,H.;El-Mashad,A.-R.;El-Bahrawy,H.;El-Gohary,T.;El-Barbary,A.;Aly,H.Humanrecombinant erythropoietin in asphyxia neonatorum: Pilot trial. *Pediatrics* **2010**, *125*, e1135–e1142.	45 infants (38-42 weeks' gestation), 3 groups: normal (*n* = 15), HIE with conventional treatment (*n* = 15), or HIE with EPO treatment (*n* = 15)	2500 U/kg daily for 5 days	s.c	When 2-weeks-old for HIE infants, EPO decreased nitric oxide concentration and breakthrough seizures compared to conventional treatment. When 6-month-old for HIE infants, EPO decreased neurologic and developmental abnormalities. The HIE-erythropoietin group received fewer transfusions of packed red blood cells during their hospital stays. Neonates tolerated the high dose of human recombinant erythropoietin without any side effects specific to its use.
Avasiloaiei, A.; Dimitriu, C.; Moscalu, M.; Paduraru, L.; Stamatin, M. High-dose phenobarbital or erythropoietin for the treatment of perinatal asphyxia in term newborns. *Pediatr. Int.* **2013**, *55*, 589–593.	67 infants (≥37 weeks' gestation), 3 HIE groups treated with EPO & supportive care (*n* = 22), or phenobarbital (*n* = 22) or supportive care alone (*n* = 23)	1000 IU/kg Daily for 3 days	s.c	When 18-months-old, neurodevelopmental delay was lower in both the EPO and phenobarbital treatment groups, although the differences were not statistically analysed.
ElShimi,M.S.;Awad,H.A.;Hassanein,S.M.;Gad,G.I.;Imam,S.S.;Shaaban,H.;ElMaraghy,O. Single dose recombinant erythropoietin versus moderate hypothermia for neonatal hypoxic ischemic encephalopathy in low resource settings. *J. Matern. Fetal Neonatal Med.* **2014**, *27*, 1295–1300.	45 infants (>36 weeks' gestation), HIE/EPO (*n* = 15), HIE/hypothermia (*n* = 15), normal (*n* = 15)	1500 U/kg single dose	s.c	When 3-months-old, no significant differences in neuromuscular function nor brain MRI score Haemoglobin concentration, platelet count, alanine aminotransferase, creatinine levels and urine output showed non-significant difference between groups.
Malla,R.R.;Asimi,R.;Teli,M.A.;Shaheen,F.;Bhat,M.A.Erythropoietinmonotherapyinperinatalasphyxia with moderate to severe encephalopathy: A randomized placebo-controlled trial. *J. Perinatol.* **2017**, *37*, 596–601.	100 infants (≥37 weeks' gestation), HIE/EPO (*n* = 50), HIE/placebo (saline, *n* = 50)	500 U/kg alternate days for a	s.c	When 19-months-old, the EPO-treated group had a lower risk of cerebral palsy. EPO also decreased death.

		total of 5 doses		The incidence of adverse outcomes was similar between the two groups. Hypotension, hepatic dysfunction, prolonged coagulation and necrotizing enterocolitis were similar between the two groups. Although red blood cell indices (haemoglobin, red blood cell count and reticulocyte count) were elevated in the treatment group on day 10 of life, these normalized by 1 month of age and no complications were seen. There were no patients with hypertension, polycythaemia or thrombosis secondary to EPO.
Baserga,M.C.;Beachy,J.C.;Roberts,J.K.;Ward,R.M.;DiGeronimo,R.J.;Walsh,W.F.;Ohls,R.K.;Anderson,J.; Mayock, D.E.; Juul, S.E.; et al. Darbepoetin administration to neonates undergoing cooling for encephalopathy: A safety and pharmacokinetic trial. *Pediatr. Res.* **2015**, *78*, 315–322.	30 infants (≥36 weeks' gestation) with hypoxic-ischemic encephalopathy (HIE), 3 groups, placebo (*n* = 10), EPO (low dose, *n* =10), EPO (high dose, *n* = 10)	2 or 10U/kg of Darbepoetin 2 doses.	i.v.	HT combined with EPO was safe. Weekly administration of darbepoetin was sufficient AEs were similar between all three study groups. The most commonly noted AEs in all of the groups included: hypotension, altered renal function, and pulmonary hypertension. No patients were found to have polycythaemia, neutropenia, or sepsis. Feeding difficulties and anticonvulsant therapy for seizures at the time of discharge were similar between the study groups.
Valera, I.T.; Vázquez, M.D.; González, M.D.; Jaraba, M.P.; Benitez, M.V.R.; Morano, C.C.; Laso, E.L.; Cabanas, J.M.G.; Quiles, M.J.P. Erythropoietin with hypothermia improves outcomes in neonatal hypoxic ischemic encephalopathy. *J. Clin. Neonatol.* **2015**, *4*, 244–249.	15 infants (≥36 weeks' gestation) with HIE and treated with EPO and moderate hypothermia	400 U/kg alternate days for 2 weeks.	i.v.	When 18-months-old, 80% survival with no neurodevelopmental disability. Unfortunately, no control group. There were no serious adverse events such as major venous thrombosis, polycythaemia (haematocrit >60%) or retinopathy. Nor negative hematopoietic side-effects were observed.
Rogers, E.E.; Bonifacio, S.L.; Glass, H.C.; Juul, S.E.; Chang, T.; Mayock, D.E.; Durand, D.J.; Song, D.; Barkovich, A.J.; Ballard, R.A.; et al. Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatr. Neurol.* **2014**, *51*, 657–662.	24 infants (≥37 weeks' gestation) with HIE and treated with EPO and moderate hypothermia; 250U/kg EPO(*n*=3), 500(*n*= 6), 1000 (*n* = 7), 2500 (*n* = 8)	250 to 2500 U/kg alternate days up to 6 doses	i.v.	When 8-34-months-old, neurodevelopmental delay was lower compared to treatment with hypothermia alone. However, study statistically underpowered to detect a statistical difference. There were no neonatal deaths, and the frequency of systemic complications was not statistically different from that reported in historical controls who received hypothermia alone.
Mulkey,S.B.;Ramakrishnaiah,R.H.;Mckinstry,R.C.;Chang,T.;Mathur,A.M.;Mayock,D.E.;VanMeurs,K.P.; Schaefer, G.B.; Luo, C.; Bai, S.; et al. Erythropoietin and brain magnetic resonance imaging findings in hypoxic-ischemic encephalopathy: Volume of acute brain injury and 1-year neurodevelopmental outcome. *J. Pediatr.* **2017**, *186*, 196–199.	44 infants (≥36 weeks' gestation) with HIE; In addition to treatment with moderate hypothermia, treated with EPO(*n*=20) or saline(*n*=24)	1000 U/kg 3-4-5 doses (11 infants received 3 doses, 8	i.v.	Statistically significant lower volume of acute brain injury in the EPO-hypothermia-treated group compared with the saline-hypothermia group

		4 doses, and 1 infant 5 doses)		
Wu,Y.W.;Mathir,A.M.;Chang,T.;McKinstry,R.C.;Mulkey,S.B.;Mayock,D.E.;Van Meurs,K.P.;Rogers,E.E.; Gonzalez, F.F.; Comstock, B.A.; et al. High-dose erythropoietin and hypothermia for hypoxic-ischemic encephalopathy: A phase II trial. *Pediatrics* **2016**, *137*.	50 infants (≥36 weeks' gestation) with HIE; In addition to treatment with moderate hypothermia, treated with EPO(*n*=24)or saline(*n*=26)	1000 U/kg 5 doses	i.v.	Significantly less brain injury at 5 days-of-age, and better 12-month motor outcomes, in the EPO-hypothermia-treated group compared with the saline-hypothermia group No adverse events were attributed to Epo. Expected adverse events were common and evenly distributed, except for a higher rate of sepsis in the placebo group. No patients in either group developed polycythaemia. The haematocrit did not differ between groups. Serious adverse events occurred in 9 patients and were seen in both treatment groups.
Juul, S.E.; Comstock, B.A.; Heagerty, P.J.; Mayock, D.E.; Goodman, A.M.; Hauge, S.; Gonzalez, F.; Wu, Y.W. High-dose erythropoietin for asphyxia and encephalopathy (HEAL): A randomized controlled trial—background, aims, and study protocol. *Neonatology* **2018**, *113*, 331–338.	Recruiting 500 term infants with HIE; In addition to treatment with moderate hypothermia, treated with EPO or saline	1000 U/kg 5 doses	i.v.	Assessment up to 24 months-of-age. Hypothesize that EPO in combination with hypothermia reduces mortality and neurodevelopmental disability
Wang,S.Y.EffectofmildhypothermiacombinedwithVitCandEPOtherapyontargetorgandamagein children with neonatal asphyxia. *J. Hainan Med. Univ.* **2017**, *23*, 117–120.	68 infants (37-40 weeks' gestation) with HIE, 2 groups, moderate hypothermia, EPO & Vitamin C (*n* = 34), EPO & Vitamin C (*n* = 34)	500 U/kg 3 doses/week	i.v. for EPO and Vitamin C (250 mg/kg)	Mild hypothermia, EPO & Vitamin C combined more effective. This was achieved through decreased apoptosis and oxygen free radicals, and increased antioxidant capacity
Nonomura, M.; Harada, S.; Asada, Y.; Matsumura, H.; Iwami, H.; Tanaka, Y.; Ichiba, H. Combination therapy with erythropoietin, magnesium sulfate and hypothermia for hypoxic-ischemic encephalopathy: An open-label pilot study to assess the safety and feasibility. *BMC Pediatr.* **2019**, *19*, 13.	9 infants (≥36 weeks' gestation) with severe HIE, moderate hypothermia, EPO & magnesium sulphate (Mg)	300 U/kg Alternate days for 2 weeks	i.v. for EPO and Mg (250 mg/kg)	No deaths and all 9 neonates did not have any serious adverse effects