PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy

PAEAN
Preventing Adverse Outcomes of Neonatal Hypoxic Ischaemic Encephalopathy with Erythropoietin: A Phase III Randomised Placebo Controlled Multicentre Clinical Trial

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PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy

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<th>Measurement</th>
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### REFERENCES

1. **Clinical study report**
2. **Audit and inspection**
3. **Study monitoring**
4. **Protocol amendments**
5. **Reporting to regulatory bodies**
6. **Placental pathology central review**
7. **MRI**
8. **PAEAN**
9. **Preparation and administration of blinded study treatment**
10. **Erythropoietin for hypoxic ischaemic encephalopathy**
11. **Use of community-based health services**
12. **Biomarkers and pharmacokinetic studies**
13. **Adverse Events**
14. **Use of community-based health services**

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**PAEAN protocol version 2.0 dated 15 February 2016**

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## SYNOPSIS AND SCHEMA

### PROTOCOL SYNOPSIS

#### Background

Birth asphyxia and the consequent hypoxic-ischaemic encephalopathy (HIE) affects 1-3/1000 live-born infants in Australia. HIE survivors are disabled by cerebral palsy, cognitive impairment, blindness and epilepsy which impose an enormous burden on the child, the family and society. Even with hypothermia, the only currently available neural rescue therapy for HIE, 46% of term infants with moderate-severe HIE will die or survive with major neuro-developmental impairments. There is an urgent need for ‘add-on’ therapies to hypothermia, to reduce the rate and severity of neurodevelopmental impairments after HIE. Erythropoietin (Epo) is one such therapy; it has neuro-protective effects that are distinct from hypothermia, it may be additive in combination and it is safe.

#### General aim

To determine whether Epo given in conjunction with hypothermia to infants with moderate-severe HIE will improve neurodevelopmental outcomes at 2 years of age, without significant adverse effects, when compared to hypothermia alone.

#### Primary objective (endpoint)

To compare composite of death or moderate/severe disability in infants treated with either erythropoietin or control group (placebo) at 2 years of age (death and disability assessments)

#### Secondary objectives (endpoints)

To compare in infants treated with either erythropoietin or control (placebo) at 2 years of age:

- a. Death (at any time from day 1 of treatment to 2 years of age)
- b. Cerebral palsy
- c. Moderate/severe motor deficit (cerebral palsy (CP) plus GMFCS score)
- d. Cognitive deficit (Bayley III Cognitive Score)
- e. Need for supplementary respiratory or nutritional support
- f. Cortical visual impairment
- g. Hearing impairment status
- h. Autistic spectrum disorder (M-CHAT score)
- i. Epilepsy
- j. Costs of healthcare and service utilisation

To compare in infants treated with either erythropoietin or placebo safety, including death, up to 30 days after last study dose (frequency of selected adverse events of interest)

#### Tertiary objectives

Include the following sub-studies

- a. Pharmacokinetics
- b. Biomarkers of brain injury and inflammation
- c. General Movements Analysis at 12 weeks of age
- d. Placental pathology

#### Design

Double-blind, placebo-controlled, parallel, 2 arm randomised, phase III multicentre trial, stratified by study site and by severity of encephalopathy at study entry
**PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy**

<table>
<thead>
<tr>
<th>Population</th>
<th>The target population is newborn term or near term infants (≥ 35+0 weeks gestation) with hypoxic ischaemic encephalopathy and receiving, or planned to receive, cooling who are able to be recruited in time to allow study treatment to commence before 24 hours of age.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study treatments</td>
<td>The treatment group will receive human recombinant erythropoietin, 1000 IU/kg IV (capped at 4000 IU) on days 1, 2, 3, 5 &amp; 7 of life. The control group will receive 0.9% sodium chloride as a placebo on days 1, 2, 3, 5 &amp; 7 of life.</td>
</tr>
<tr>
<td>Assessments</td>
<td>Eligibility should be determined within 23 hours after birth using assessments including modified Sarnat criteria (See Appendix 1). Safety assessments will be performed daily while on study treatment and recorded up to 30 days after last study dose. Other assessments/collections as follows: a. Pharmacokinetic samples on days 1-7 of treatment, according to one of three regimens b. Biomarker samples prior to treatment on days 1, 2, 3 and 5 c. Digital files of aEEG monitoring and MRI, (performed at any time in the first two weeks, according to site routine) d. A video for General Movements assessment will also be taken when the child is approximately 12 weeks of age</td>
</tr>
<tr>
<td>Follow up assessments will be conducted</td>
<td>a. Every 6 months up to and including 2 years to collect resource utilisation, baby health &amp; ongoing contact data b. At 2 years of age, primary outcome will be assessed using GMFCS, neurological examination, Bayley III, M-CHAT and a paediatric health status questionnaire c. From 2 to 8 years of age using age appropriate instruments and assessments</td>
</tr>
<tr>
<td>Statistical considerations</td>
<td>The sample size of 150 per treatment group is large enough to detect a 19% absolute risk reduction in the combined endpoint of death or severe/moderate motor/cognitive deficit assuming a control event rate of 46%, (decrease from 46% to 27%) and allowing for a 10% non-compliance/lost to follow-up rate with 90% power and a two-sided Type I error of 0.05. Three hundred infants will be recruited. Each infant will be followed for 24 months to assess the primary and secondary outcomes.</td>
</tr>
</tbody>
</table>
PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy

Study Schema

Eligibility
Infants ≥35+0 weeks gestation with moderate or severe HIE

IV erythropoietin
1000 IU/kg (capped at 4000 IU)
days 1, 2, 3, 5, & 7

Stratification
- By site
- Modified Sarnat criteria

IV placebo
0.9% sodium chloride
days 1, 2, 3, 5, & 7

R 1:1

Endpoint
At 2 years of age
Composite death or moderate severe motor or cognitive deficit
1 BACKGROUND

Hypoxic-ischaemic encephalopathy (HIE), resulting from significantly reduced blood flow and oxygen availability to a baby’s brain near the time of birth, occurs in 1-3 per 1000 births. (1) Meta-analysis of published trials indicates that induced hypothermia reduces the combined rate of death or major neurodevelopmental disability from 61% to 46%. (2) Yet infants who received hypothermia in clinical trials still experienced unacceptably high rates of death (25%), neuromotor delay (26%) and cerebral palsy (23%) and other neurologic impairments after HIE despite hypothermia include epilepsy (12%) blindness (6%) and deafness (4%). (2)

1.1 Erythropoietin’s neuroprotective properties may be additive with therapeutic hypothermia.

Erythropoietin (Epo) is a glycoprotein known for promoting proliferation and differentiation of erythroid precursors and treatment of anaemia. However, Epo has other actions related to response and repair of cell injury. Epo binding to Epo receptor (Epo-R) leads to activation of the phosphatidylinositol 3-kinase (PI3K)/Akt, Janus kinase 2 (JAK2)/STAT5, and extracellular signal-regulated kinase ERK1/2 pathways. Akt limits inflammation, (3) decreases apoptotic cell death and increases angiogenesis (4) while STAT5 plays a role in cell survival. (5) The ERK pathway has anti-apoptotic and anti-inflammatory effects in vitro and is critical in neurogenesis and cell fate commitment in the central nervous system (CNS). (6, 7) In addition to anti-apoptotic (8-10) and anti-inflammatory properties (11-13), Epo also increases anti-oxidant activity (14, 15) and reduces excitotoxic cell injury. (16, 17)

Although Epo signalling in the CNS is incompletely understood, several lines of evidence indicate important roles for Epo in the context of brain injury and development. First, Epo has neuroprotective and reparative effects in the CNS. (18) Second, Epo-R is expressed by most cell types in the developing CNS (19, 20), including neuronal progenitor cells, (21) subsets of mature neurons (22), astrocytes (23), oligodendrocytes (24), microglia (25) and brain endothelial cells. (21, 26) Third, both Epo and Epo-R are expressed in neurons and astrocytes during fetal life. (27) Finally, following hypoxic-ischaemic (HI) insult, there is increased expression of Epo and Epo-R in neurons, astrocytes and microglia via hypoxia-mediated stabilisation of hypoxia-inducible factor-1α. (19, 20, 28)

In vitro, Epo increases proliferation of neuronal precursor cells and directs stem cells to differentiate into neurons. (29, 30) In several in vitro and in vivo models of brain injury, Epo enhances neurogenesis and angiogenesis that are likely required for repair and long-term improvement in brain function. (31-36) Epo stimulates production of vascular endothelial growth factor (VEGF) known for angiogenic and neurogenic effects. (21) Epo protects neuronal progenitor cells from lipopolysaccharide (LPS) and HI injury, (37, 38) and protects oligodendrocytes and improves white matter survival assessed by MRI and pathologic analysis. (39, 40) In summary, there is strong evidence for Epo protective mechanisms in the brain. Many effects and mechanisms of Epo action, in particular its regenerative action, are distinct from those of hypothermia and support an additive benefit of combined therapy.

1.2 Pre-clinical studies indicate multiple doses of Erythropoietin prevent brain injury

Over 68 pre-clinical animal studies testing exogenous Epo for HI brain injury have produced convincing histologic and functional evidence for benefit. (18, 41-43) High-dose Epo administration in rodents results in a 40-75% decrease in the size of infarction. (44-46) Among 1,026 experimental treatments for acute stroke, Epo scored in the top 10 based on the Stroke Therapy Academic Industry Roundtable (STAIR) ratings of evidence supporting the effectiveness of experimental treatments. (46) Low dose Epo (200 U/kg) used to treat anaemia does not raise CSF Epo concentrations. (47) Although only 1-2% of circulating Epo crosses the blood brain barrier,
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higher doses of Epo have been shown in rats, primates and humans to achieve significant elevations in CSF Epo. (48, 49) This is particularly true in the setting of HI due to increased permeability of the blood brain barrier. (49-51)

Sixteen pre-clinical studies specifically target neonatal HI brain injury. Pre-treatment with Epo prior to HI injury is neuroprotective in neonatal rodents. (52) Al Colditz has reported that a single dose of Epo administered with global hypoxia-ischaemia in a rat pup model reduced neuronal apoptosis and protected astrocytes and oligodendrocytes. (18) Treatment after neonatal HI brain injury reduces infarct volume and improves short-term spatial memory (53); reduces sensory neglect and motor asymmetry (45), and decreases hemispheric brain loss 6 weeks after injury, with increased neurogenesis near the site of injury. (54) Multiple Epo doses administered over 1-2 weeks produce even more long-lasting improvements. (33) Neonatal rats that received 3 doses of Epo over 7 days following injury demonstrated better cognitive function and brain volumes than rats given a single dose, especially when outcomes were assessed at later time points (i.e. 3 months). (54) The cumulative dose was smaller in the 3-dose than in the single dose regimen, suggesting that neuro-protection and repair are dependent on a prolonged course of administration.

Multiple doses of Epo after experimental HI brain injury in newborn rodent models reduce infarct volume in a dose-dependent manner. (55) In a non-human primate model of HIE, Juul found increased white matter connectivity on diffusion tensor imaging, improved survival without moderate-severe CP, preserved cerebellar growth rates, and improved many neurocognitive behavioural scores in animals that received multiple doses of Epo + hypothermia compared to control animals, without adverse effects. (56) Repeated doses of Epo initiated as late as 2 days after neonatal HI injury and continued until 13 days after injury improved behavioural sensorimotor performance, enhanced neurogenesis and oligodendrogenesis, and reduced injury and enhanced reorganisation within white matter in rodents. (16, 38) There is, therefore, strong pre-clinical evidence that treatment with Epo might shift the distribution of injury toward a less severe pattern of outcomes. In summary, multiple doses of Epo, with the first dose given as late as 2 days after HI injury, are safe and provide neuro-protection in rodent and primate models of HIE.

1.3 Preliminary data from clinical studies in term HIE encourage further research

Two randomised controlled trials (RCTs) suggest that high-dose Epo may improve neuro-developmental outcome. Elmahdy et al administered 5 daily doses of Epo 2500 IU/kg to 15 infants, starting up to 24 hours after birth. (57) Compared to 15 placebo controls, those who received Epo had improved EEG backgrounds, reduced biomarkers of oxidative stress at 2 weeks, and better neuro-development at 6 months. Zhu et al administered Epo 300-500 U/kg on alternate days to 84 infants for 2 weeks, starting up to 48 hours after birth. (49) Compared to placebo controls, infants who received Epo were less likely to die or have moderate to severe disability at 18 months of age (44% vs. 25%, P=0.02). These studies were limited by their small size and by not combining Epo with hypothermia, which is now standard of care for moderate-severe HIE. While the evidence may be promising, there is currently insufficient evidence to support the use of Epo in HIE. We will conduct a multicentre randomised, placebo controlled, double-blind trial of high-dose Epo for HIE.

The PAEAN Study

1.4 Erythropoietin dose selection for this study

Pharmacokinetic data and dose selection resulted from the NEAT pilot trial. The NEAT pilot trial (48) tested the safety and pharmacokinetics of Epo + hypothermia in HIE in a 5 centre, open label, dose escalation study of 4 doses of Epo IV: 250 (N=3), 500 (N=6), 1000 (N=7) and 2500 U/kg (N=8). All infants were treated with hypothermia within 6 hours of birth and received up to 6 Epo doses, given every 48 hours. The first dose was administered within 24 hours. There were no deaths or SAE’s. No patients experienced polycythaemia; mean haematocrit decreased from 45.6 to 41.5 over the course of the study. Co-morbidity rates were no different in Epo treated infants

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compared to historic controls who received hypothermia alone. In the pilot trial, Epo followed nonlinear pharmacokinetics (Figure 1).

Steady-state plasma Epo levels were attained by the 2nd dose for all 4 dosages, and peak and trough concentrations were stable across doses (Figure 1). Plasma Epo concentrations demonstrated limited variability across individuals; average coefficient of variation for Cmax was 26%.

In a rodent model, multiple doses of Epo with mean AUC range 117,677 - 140,331 U/h/L and mean Cmax range 6,224 U/L - 10,015 U/L afford the greatest neuroprotection. (58, 59) In the (human) pilot study, Epo 1000 U/kg administered together with hypothermia produced drug exposure levels (AUC 131,054 ± 17,083 U/h/L and Cmax 13,780 ± 2,674 U/L) that most closely mimic these target neuroprotective levels (Table 1).

In contrast, Epo 500 U/kg produced insufficient plasma elevations, and Epo 2500 U/kg produced AUC and Cmax values that exceeded the optimal neuroprotective range by 3-fold. Although the upper safety limit of Epo is unknown, preclinical data suggest too much Epo may lead to diminished efficacy (58) and extremely high doses may be harmful. (60) We therefore propose to test Epo 1000 IU/kg/dose (capped at 4000 IU), the dose that establishes Epo levels within the optimal neuroprotective range in human infants receiving hypothermia, while minimising risks associated with giving excessive Epo.

1.5 Understanding and monitoring the safety of Erythropoietin

Epo side effects reported in adults include hypertension, thrombosis, red cell aplasia, myocardial infarction, stroke, congestive heart failure, seizures, tumour progression and increased death, but none of these have been reported in neonates. An adult ischaemic stroke trial of Epo reported increased mortality in patients who received tPA; however pathogenetic mechanisms of neonatal global HIE are completely different from adult atherosclerotic thromboembolic stroke, and tPA is not used in neonates. In the period 1991-2006, over 2400 preterm newborns in 30 randomised controlled trials of Epo for anaemia of prematurity received Epo 70 to 5000 IU/kg/week (35–750 IU/kg/dose) for 2 weeks to several months, with minimal safety concerns. (61) Concern that chronic Epo therapy in preterm infants < 32 weeks' gestation could increase the risk of retinopathy

Table 1. Epo pharmacokinetics in infants with HIE undergoing hypothermia

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>250 U/kg (n=3)</th>
<th>500 U/kg (n=6)</th>
<th>1000 U/kg (n=7)</th>
<th>2500 U/kg (n=8)</th>
<th>Target level</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC ([U*h]/L)</td>
<td>18426 ± 8976</td>
<td>50306 ± 7426</td>
<td>131,054 ± 17,083</td>
<td>328002 ± 61945</td>
<td>117,677-140,331</td>
</tr>
<tr>
<td>Cmax (mU/mL)</td>
<td>3156 ± 1615</td>
<td>7046 ± 814</td>
<td>13,780 ± 2,674</td>
<td>33316 ± 7377</td>
<td>6,224-10,015</td>
</tr>
<tr>
<td>t ½ (h)</td>
<td>7.6 ± 6.9</td>
<td>7.2 ± 1.9</td>
<td>15.0 ± 4.5</td>
<td>18.7 ± 4.7</td>
<td></td>
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</tbody>
</table>

In the (human) pilot study, Epo 1000 U/kg administered together with hypothermia produced drug exposure levels (AUC 131,054 ± 17,083 U/h/L and Cmax 13,780 ± 2,674 U/L) that most closely mimic these target neuroprotective levels (Table 1).
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of prematurity and skin haemangiomas has not been confirmed in prospective trials, and these are
not concerns for term infants who are the subjects of this study. None of the neonatal high-dose
Epo neuroprotection studies have led to any safety concerns. Published (48, 49, 57) and currently
recruiting studies of term brain injury and 3 studies of preterm brain injury (62-64) have included
over 405 newborns who received 3-7 doses of Epo ranging from 300–3000 U/kg/dose, with no
adverse events. The NEAT pilot trial (N=24) is the only published study that has evaluated Epo +
hypothermia in HIE. (48) It found no apparent safety issues at any dose of Epo. We hypothesise
that Epo + hypothermia in PAEAN will confirm clinical safety of this combinatorial therapy.

1.6 Societal cost of HIE and potential cost-effectiveness of Erythropoietin

There are substantial costs associated with both the initial hospitalisation and subsequent medical
care of children with HIE. In the US, each year HIE introduces an economic burden of $1.9 billion
lifetime costs due to CP alone. (65) Expenditures related to cognitive dysfunction, epilepsy, and
vision loss resulting from HIE are likely to be equally substantial. Hypothermia has been shown to
be economically appealing in comparison to standard care. (66) Neonatal therapies designed to
prevent neurodevelopmental impairment often entail significant costs early in the clinical course,
with positive economic benefits occurring later in life. The balance of resource utilisation and later
financial benefit can only be estimated through formal economic evaluation, ideally using
prospectively collected data in a RCT.

1.7 Summary

Birth asphyxia and the consequent hypoxic-ischaemic encephalopathy (HIE) causes over 700,000
yearly neonatal deaths worldwide. Of the HIE survivors, over 400,000 are disabled by cerebral
palsy, cognitive impairment, blindness and epilepsy which impose an enormous burden on the
child, the family and society (67). Because many of the antenatal and intrapartum events that lead
to HIE are highly unpredictable and in some cases unknown, it is very unlikely that HIE will ever be
completely preventable, even in countries with high health care resources. Even with hypothermia,
the only currently available neural rescue therapy for HIE, 46% of term infants with moderate-
severe HIE will die or survive with major neuro-developmental impairments. There is an urgent
need for ‘add-on’ therapies to hypothermia, to reduce the rate and severity of neurodevelopmental
impairments after HIE. Erythropoietin (Epo) is one such therapy as it has neuro-protective effects
that are distinct from hypothermia and may be additive in combination.

We hypothesise that Epo given in conjunction with hypothermia to infants with moderate-severe
HIE will improve neurodevelopmental outcome at 2 years of age, without significant adverse
effects. This outcome would reduce mortality and long-term morbidity, and produce significant
societal cost savings.
2 AIM AND OUTCOMES

2.1 General aim
The primary aim is to assess the effect of Epo on death or neurodevelopmental outcome in term infants receiving hypothermia for hypoxic-ischaemic encephalopathy (HIE) by conducting a randomised controlled trial.

2.2 Outcomes

2.2.1 Primary outcome
The primary outcome is a composite of death or survival with moderate/severe developmental deficit (motor or cognitive) based on standardised neurological and developmental assessments measured at 2 years age (22 – 26 months of age).

Moderate or severe developmental deficit will consist of the following components:
- Motor deficit (a combination of cerebral palsy (CP) plus the Gross Motor Function Classification Scale (GMFCS) score)
- Cognitive deficit (Bayley Scales of Infant Development III (BSDIII) score)

2.2.2 Secondary outcomes
To compare at 2 years:
- Death (at any time from day 1 of treatment to 2 years of age)
- Cerebral palsy
- Motor deficit (CP plus GMFCS)
- Cognitive deficit (BSDIII cognitive score)
- Supplementary respiratory support
- Nutritional support
- Cortical visual impairment status
- Hearing impairment status
- Autistic spectrum disorder (M-CHAT score)
- Epilepsy
- Costs of health care and/or service utilisation (Medicare, PBS) up to 2 years of age

At 30 days after last study treatment:
- Frequency of selected adverse events of interest, including deaths, during and up to 30 days post study treatment

At 8 years of age
- Motor and cognitive deficit to 8 years of age, using age appropriate instruments and assessments. Note this is subject to additional funding being granted.

2.2.3 Tertiary outcomes
1. Shifted distribution of overall severity across the following 4 domains: 1) normal; 2) mild motor or cognitive deficit; 3) moderate/severe motor or cognitive deficit; and 4) death

2. Sub-studies
- Pharmacokinetics
- Biomarkers of injury and brain inflammation
- General Movements Analysis at approximately 12 weeks of age
- Placental pathology
3 DESIGN

This study is a randomised, phase III, double-blind, placebo-controlled, parallel group trial. Participants will be centrally randomised through the NHMRC CTC. Treatment allocation will be balanced using minimisation for the following characteristics:

- study site
- severity of encephalopathy (moderate vs severe, as measured by modified Sarnat score between 1 and 6 hours of age, see Appendix 1)

Participants will be allocated to the treatment group in a ratio of 1:1.

4 SUBJECT POPULATION

Participants must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for this trial. There will be no exceptions made to these eligibility requirements at the time of registration. All enquiries about eligibility should be addressed by contacting the CTC or Study Chair or delegate (if outside business hours), prior to registration.

4.1 Target population

Term or near-term infants (≥ 35+0 weeks of gestation) with moderate or severe HIE in which hypothermia treatment (controlled whole-body cooling to a target temperature, either adjusted manually or with a device) is initiated by 6 hours of age.

4.2 Inclusion criteria

1. Male or female infants born ≥ 35+0 weeks gestation and able to be randomised less than 23 hours after birth.
2. One or more of the following indications of perinatal depression:
   - Apgar ≤ 5 at 10 minutes after birth OR
   - receiving ongoing resuscitation e.g. assisted ventilation (positive pressure ventilation or CPAP) or chest compressions at 10 minutes after birth OR
   - on cord blood or arterial or venous blood obtained at < 60 minutes after birth,
     - pH less than 7.00 OR
     - base deficit ≥ 12.0 mmol/L.
3. Moderate to severe encephalopathy, defined between one and six hours after birth by one or both of the following
   - 3 out of 6 modified Sarnat criteria (see Appendix 1) indicating moderate/severe encephalopathy
   OR
   - 2 out of 6 modified Sarnat criteria between one and six hours after birth, plus seizure(s) requiring anticonvulsant treatment (diagnosed either clinically or using EEG monitoring) at any time prior to randomisation
4. Hypothermia treatment initiated by 6 hours of age; i.e. controlled whole-body cooling planned to continue for 72 hours, to a target temperature (adjusted manually or with a device) and subsequent controlled re-warming
5. Study treatment planned to start within 24 hours after birth (as soon as feasible after randomisation)
6. At least one parent ≥ 18 years of age
7. Anticipated ability to collect primary endpoint at 2 years of age
8. Signed, written informed parental consent

4.3 Exclusion criteria

1. Contraindications to investigational product
2. Indication prior to randomisation for erythropoietin or any other erythropoietic stimulating agent to be given during the first two weeks of life
3. Severe intrauterine growth restriction (birth weight less than 1800g)
4. Suspected major chromosomal or congenital anomalies
5. Head circumference < 3rd centile below the mean for gestation and gender (see appendix 2)
6. Infant for whom imminent withdrawal of care is being planned

4.4 Consent

Written informed consent must be signed and dated by the parent(s), and signed and dated by the Investigator or his/her delegate, prior to randomisation and study treatment. In circumstances where written informed consent cannot be obtained in time to allow randomisation by 23 hours of age, telephone parental consent witnessed by a person who is independent of the study is acceptable, provided the study personnel obtaining consent are satisfied that the consent is informed and freely given. Confirmation of consent in the form of a signed consent form should be obtained as soon as feasible after parent(s) arrive at the study site.

Any family that wishes to withdraw their baby from the trial may do so, without giving a reason and without any change in any other aspect of treatment. Permission will be sought from parents of any baby who is withdrawn from the study after randomisation and before or after the intervention is administered to follow the baby’s progress and allow collection of outcome data. Parents may choose to withdraw this permission as well. Parents may withdraw their consent for provision of specific information, such as economic data, while continuing to participate in the clinical study.

4.5 Registration and Randomisation

Participants must meet all of the inclusion criteria and none of the exclusion criteria to be eligible for this trial. There will be no exceptions made to these eligibility requirements at the time of registration. All enquiries about eligibility should be addressed by contacting the CTC, or Study Chair or delegate (if outside business hours), prior to registration.

Treatment should be planned to start within 24 hours after birth. Registration and randomisation should be done according to the instructions in the Study Manual only after all screening assessments have been performed and the responsible investigator has both verified the participant’s eligibility and completed the randomisation form.

Once the randomisation process has been completed, the participant will be assigned a treatment arm and participant study number. Emailed confirmation that randomisation has occurred will be provided to the site. If twin babies are eligible, the babies will randomised separately.

Participants may not be registered/randomised more than once to this trial.

5 TREATMENT PLAN

5.1 Administration of study treatments

Participants will receive either Epo 1000 IU/kg IV (capped at 4000 IU) or an equal volume of 0.9% sodium chloride (NS). The study intervention will be infused intravenously over 5 min, followed by a 0.9% sodium chloride flush and administered on days 1, 2, 3, 5, and 7 of age or up until death or hospital discharge if prior to 5th dose.
PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy

Treatment is to be administered after randomisation and within 24 hours after birth, then for four subsequent doses as close as possible to 24, 48, 96 and 144 hours after the first dose.

Cooling will be provided according to the site routine. Guidelines/recommendations are provided in the study manual.

5.2 Dose modifications
No dose modifications or dose titrations are permitted within the study.

5.3 Treatment discontinuation
Study treatment will be permanently discontinued for any of the following reasons:
- Unacceptable toxicity as determined by the parent or site investigator. Examples of unacceptable toxicity include hypersensitivity reaction to the study drug or any of its excipients.
- Delay of any day’s treatment for >24hrs due to treatment-related adverse events. For delays >24hrs for any reason, please contact the CTC to discuss treatment continuation.
- The investigator determines that continuation of treatment is not in the infant’s best interest.
- Occurrence of an exclusion criterion, e.g. a decision has been made for withdrawal of care or new diagnosis of a major congenital or chromosomal anomaly has been made.
- Required use of a concomitant treatment that is not permitted, as defined in section 5.5.4.
- The parent declines further study treatment, or withdraws their consent to participate in the study.
- A participating infant has made a sufficiently good recovery that discharge from hospital occurs before 7 days of age.

The reasons for discontinuing treatment will be documented in the participant’s medical record.

5.4 Rechallenge
If a participant experiences a suspected mild drug related adverse event or a moderate or severe adverse event where there is a low index of suspicion that the event is related to study drug, the investigator can interrupt the study medication for up to one day. The CTC should be contacted to discuss continuation of the participant in the study for any delays longer than one day. If the reaction reappears, then the study medication is to be discontinued permanently.

5.5 Concomitant Medications/Treatments

5.5.1 Recommended
No other medications or treatments are specifically recommended in this study.

5.5.2 Permitted
Participants in the trial may receive any medications required for treatment of complications or comorbid conditions associated with HIE, including (but not limited to) antibiotics, anticonvulsants, inotropes, vasodilators (e.g. nitric oxide), and sedatives.

5.5.3 Use with caution
There are no medications that should specifically be used with caution in this study.

5.5.4 Prohibited
Off-protocol use of erythropoietin or any other erythropoietic stimulating agent during the first two weeks of life is not allowed.
5.5.5 Concomitant medication reporting
Concomitant medications will not be recorded during the study, except for anticonvulsants and inotropes, and medications taken at the time of a serious adverse event.

5.6 Compliance
Administration of study drug will be recorded in the hospital drug chart in accordance with local hospital policy.

5.7 Unblinding
Blinding of families to treatment allocation will be maintained throughout the trial. Unblinding is not generally necessary for the management of a participant with an adverse event and, as it has an impact on the study’s validity, it is strongly discouraged. However, if required it should be done centrally after discussion with the Study Chair using the emergency unblinding number at the front of this protocol.

5.8 Subsequent treatment
Treatment after discontinuation of study treatment is at the discretion of the participant’s clinician. Follow up of participants who stop study treatment should continue according to this protocol (see Section 6).
## 6 ASSESSMENT PLAN

### 6.1 Schedule of assessments

<table>
<thead>
<tr>
<th>Assessments</th>
<th>Screening</th>
<th>On treatment</th>
<th>Hospital discharge</th>
<th>12 weeks</th>
<th>Follow up 6 monthly</th>
<th>Follow up at 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
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<tr>
<td>Inclusion/Exclusion criteria review</td>
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<tr>
<td>Modified Sarnat(^1)</td>
<td>X (1-6 h)</td>
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<tr>
<td>Request placental slides, pathology report</td>
<td>X</td>
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<tr>
<td>PK blood sample(^2)</td>
<td>X</td>
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<tr>
<td>Biomarker sample(^2, 4)</td>
<td>D1, 2, 3, 5</td>
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<tr>
<td>Haematology, coagulation profile, biochemistry(^3)</td>
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<td>aEEG(^4)</td>
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<tr>
<td>Neuroimaging MRI</td>
<td>X</td>
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<td>Dubowitz score</td>
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<tr>
<td>Record of anticonvulsant medications</td>
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<tr>
<td>Respiratory assessment</td>
<td>X X</td>
<td>Up to 30 days after last dose</td>
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<tr>
<td>Serious adverse events</td>
<td>X X</td>
<td>Up to 30 days after last dose</td>
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<tr>
<td>Record/confirm contacts for family</td>
<td>X X</td>
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<tr>
<td>General Movements video (parents)</td>
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<tr>
<td>Questionnaire: health services (parents)</td>
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<tr>
<td>Questionnaire: Warner IDEA-FS (parents)(^5)</td>
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<tr>
<td>M-CHAT (parents)</td>
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<tr>
<td>GMFCS, Neurological examination, Bayley III(^6)</td>
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<td>PBS, Medicare service utilisation (Australian participants)</td>
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</table>
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1. Modified Sarnat assessments are only required on Days 2-7. The screening Sarnat assessment will suffice for Day 1.
2. Refer to Biological Sampling Handbook for procedures
3. Haematology, coagulation and biochemistry results (performed per local practice) can be collected in 24 hour epochs, ideally before each study treatment is administered.
4. A biomarker sample and aEEG record are required prior to first treatment dose
5. Warner IDEA-FS is only required at 2 years if parents do not present for clinic visit.
6. Where parents do not present for clinic visit, a parent questionnaire on health outcomes is to be sent to the parents for completion.

* These materials may be collected at any time prior to hospital discharge.

6.2 Assessment phase definitions and special circumstances

6.2.1 Screening
All screening procedures must be performed within 23 hours after birth, prior to randomisation

6.2.2 On treatment
Assessments during treatment consist of determination of any possible treatment-related serious adverse events, recording of anticonvulsant medication use and respiratory support. Serum biochemistry (including liver and renal function assessment), haematology and coagulation profile should be performed according to site routine for babies receiving hypothermia for HIE, plus any additional sampling that is deemed necessary for clinical reasons.

Safety assessments will be performed daily while on study treatment.

Specified study sites will collect blood samples for pharmacokinetic and biomarker studies. Wherever feasible, this will be coincident with routine sampling from indwelling sampling lines, e.g. UAC or peripheral arterial line.

Sample for biomarker studies is two filled blood spots (approximately 0.1 mL) on a filter paper neonatal screening card. Collect 2 blood spots just prior to dose administration on day 1 (i.e. at Baseline), 2, 3 and 5, for a total of approximately 0.4 mL. Refer to the Biological Sampling Handbook for collection and processing procedures.

For pharmacokinetic studies, 0.5 mL of blood will be collected at five pre-specified times throughout the treatment period, for a total of approximately 2.5 mL. To minimise the amount of blood taken from each baby, there are three pharmacokinetic sampling regimens defined in the study Biological Sampling Handbook. The CTC will assign each baby to one of the three sampling regimens. The days and times of each sample collection vary between the regimens, however the number and volume of samples are the same.

Respiratory assessments will be collected daily whilst on treatment (maximum FiO2, inhaled nitric oxide, type of respiratory support), and after discharge (any respiratory support or supplemental oxygen at 30 days after last study dose) as well as at 2 years of age (at the 2 year assessment).

Neuro-imaging (MRI scans): Scanning will be done according to each unit’s routine and according to clinical need for babies receiving hypothermia for HIE. It is anticipated that cerebral MRI will be performed for most study babies (usually between days 4 to 10) according to each site’s clinical routine and the clinical condition of the baby. Some will be on treatment and some after the study treatment. A digital copy (in anonymous form) will be sent to the coordinating centre. Refer to Study Manual for further information.

Dubowitz score (68, 69) will be obtained on day 7 of age.
aEEG monitoring: It is anticipated that most babies in the study will have aEEG monitoring, generally for the duration of cooling and during the rewarming period, depending on site clinical routine for babies receiving therapeutic hypothermia. Data will be collected if available.

Request for placental slides and pathology reports and aEEG monitoring may be performed once treatment has commenced, according to normal site procedures.

### 6.2.3 End of treatment

The reason for ceasing study treatment will be collected at the end of treatment (e.g. completed protocol-prescribed regimen, discharged, deceased, withdrawn from study).

### 6.2.4 Follow up at 12 weeks of age

#### Post discharge safety assessment

Parents will be contacted by telephone at approximately 12 weeks to determine any adverse events (including death or hospital readmission) occurring within 30 days after the last dose of study treatment and to determine at the 30 day time point whether the baby was receiving oxygen or respiratory support. The family’s contact details will also be confirmed.

#### General Movements analysis at 12 weeks

General Movements analysis is a screening assessment which has very good sensitivity and specificity for prediction of later cerebral palsy (70). Parents will be provided before discharge with verbal and written instructions as to how to prepare a three to five minute video recording of their infant (using a hand-held video camera or smart phone) for General Movements analysis. The central reading and evaluation will not be provided in “real time” or used to direct assessment or management of the child. For any sites using General Movements analysis for routine clinical assessment, the video can be recorded by clinical staff.

### 6.2.5 6 monthly follow up

To facilitate good follow up, 6 monthly contact by phone and/or post will be maintained with the families or their nominated relatives and friends to confirm the family’s contact details in case of a change of address. Simple questionnaires will be administered orally to update contact details and assess health (Warner IDEA-FS questionnaire) and in writing for associated service utilisation (parent reported health service questionnaire). The latter questionnaire will record the range of health services that have been accessed for the child in the prior 6 months since discharge from hospital. Parents will also be asked, for each service used, the number of occasions of use in the last 6 months. For hospital admissions of at least 1 night, parents will also be asked for hospital name, length of stay and broad reason for admission. These are the minimum data required to cost service use using available standard costs of care.

### 6.2.6 2 year follow-up

Parents will be asked to attend a 2 year (22 – 26 months) clinic visit with their child to perform the

- Bayley Scales of Infant Development III (BSDIII)
- Neurological exam for CP
- GMFCS
- Modified Checklist for Autism in Toddlers (M-CHAT) – parent assessed
- Paediatric review of child health (for epilepsy, respiratory assessment etc). Respiratory assessment will consist of the need for supplemental respiratory support.

At this point if parents cannot attend, medical records will be reviewed for prior paediatric and neurological assessments (or by parent questionnaire if no hospital assessments are available).

Methods of follow-up:
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The parents of infants who stop study treatment prior to the time recommended in the protocol will be asked to continue follow-up assessments according to the protocol. If a parent wishes that their baby not undergo any clinical assessments at 2 years, they will be requested to allow their baby’s ongoing health status to be periodically reviewed via:
- phone contact
- their general practitioner
- medical records
- the national mortality registry (AIHW).

Parents will also be asked to consent to access by the Sponsor and collaborators from Deakin University to the child's Medicare (MBS and PBS) data from birth to age 2 years (Australian participants only). Medicare data includes information on all drugs, investigations, procedures and visits with certain medical practitioners that attract a Medicare subsidy.

For Australian participants who have been lost to follow-up, Medicare (Department of Human Services) may be used to provide updated contact information and the AIHW may be used to collect mortality information.

6.2.7 8 year follow-up visit

Subject to funding, the cohort will be followed up at 8 years of age for longer term health and developmental outcomes over the longer term. After 2 years, annual contact will be maintained with families and their nominated relatives and friends to confirm the family’s contact details in case of a change of address.

6.2.8 After study is closed

The study will be closed after the primary outcome objectives at 2 years have been published. 8 year follow up will continue if additional funding becomes available.
7 OUTCOMES, ENDPOINTS AND OTHER MEASURES

7.1 All-cause mortality (death from any cause)
All-cause mortality is defined as the number of deaths from any cause. The time to death is defined as the interval from the date of randomisation to date of death from any cause, or if status is unknown, censoring at the date of last known follow-up alive.

7.2 Neurodevelopmental outcomes (including death, motor or cognitive deficit) at 2 years of age
The primary outcome is a composite of death or survival with moderate/severe developmental deficit (motor or cognitive) based on standardised neurological and developmental assessments measured at 2 years age

Moderate or severe developmental deficit will comprise either moderate or severe motor or cognitive deficit defined as:

- Moderate-severe motor deficit is defined as any incident of cerebral palsy (any of quadripareisis (QP); cerebral palsy (CP); hemiparesis (HP) or diparesis (DP)) plus any level of functional impairment using the Gross Motor Function Classification Scale (GMFCS) ≥ 1.0

<table>
<thead>
<tr>
<th>Table 2. Motor deficit severity defined by CP and GMFCS function score.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Deficit</td>
</tr>
<tr>
<td>Severe</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Mild</td>
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<tr>
<td>None</td>
</tr>
</tbody>
</table>

- Moderate or severe cognitive deficit will be defined as a cognitive score ≤ 80 as assessed by the Bayley Scales of Infant Development III (BSDIII)

7.3 Secondary outcomes
The following will be determined at 2 years of age unless specified:

- Modified Checklist for Autism in Toddlers (M-CHAT). Score is positive if 2 of 6 “critical” items (Items 2, 7, 9, 13, 14, 15) or three of any of the 23 total items are abnormal

- Epilepsy is defined by a history of ≥ 2 afebrile, unprovoked seizures since discharge from the neonatal unit where PAEAN study treatment was provided and up to 2 years of age, or use of anticonvulsants at 2 years of age

- Supplementary respiratory support includes tracheostomy, ventilator, high flow nasal cannula, CPAP or oxygen dependency

- Nutritional support includes gastrostomy or nasogastric feeds

- Major cortical visual impairment

- Hearing impairment status defined as the requirement for hearing aids (either diagnosis of: Hears well or with only a little difficulty WITH a hearing aid OR Has severe hearing difficulty even with a hearing aid or hearing is not helped with an aid)
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7.4 Other exploratory measures

- Moderate to severe encephalopathy in the first 7 days after randomisation using the modified Sarnat classification

- Respiratory morbidity:
  - During treatment; dependence on respiratory support during each day of treatment (highest FiO2, low flow oxygen / supplemental oxygen, inhaled nitric oxide (highest concentration), ECMO, CPAP, high flow, ventilation)
  - At 30 days after last study dose; receiving any respiratory support (supplemental oxygen, CPAP, high flow, ventilation)

- Dubowitz score at day 7

- MRI abnormalities caused by HIE determined via analysis of images and spectroscopy results, classified by severity.

- aEEG background and seizure frequency/severity

- General Movements Analysis: normal or ‘at risk’ assessment

- Distribution of disability outcomes across four levels of severity: 1) normal; 2) mild motor or cognitive deficit; 3) moderate/severe motor or cognitive deficit; and 4) death.

7.5 Use of community-based health services

Data from parent questionnaires and the Department of Human Services (Australian participants) will be used to define pharmaceutical and medical resource usage.

7.6 Adverse Events

Frequency of selected adverse events of interest up to 30 days after the last study dose.

7.7 Biomarkers and pharmacokinetic studies

These will include exploratory studies of tissue and blood samples to identify biomarkers that are prognostic and/or predictive of response to treatment, safety and resistance to study treatment (associations of biomarkers with clinical outcomes). Studies may include, but are not limited to:

- investigating biomarkers associated with hypoxic ischaemic encephalopathy:
  - brain injury: S100B, glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), and Ubiquitin C-terminal hydrolase-L1 (UCH-L1) and Annexin A
  - inflammation: interferon-gamma (IFN-γ), IL-8, IL-10, tumour necrosis factor-α (TNF-α), TNF-β, matrix metalloproteinase-2 (MMP-2), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and monocyte chemotactic protein-1 (MCP-1)

- studies that may help to understand the placental pathologies associated with HIE

Since the identification of new biomarkers correlating with disease activity and the efficacy or safety of treatment is evolving, the definitive list of biomarkers remains to be determined.

A pharmacokinetic/pharmacodynamic substudy (serum levels of erythropoietin) will be conducted to assess:

- clinical factors (e.g. gestation, organ dysfunction, phase of cooling/rewarming) that affect pharmacokinetic variables (peak levels, clearance, etc.)
- relationship between pharmacokinetic variables and response
8 SPECIMEN COLLECTION AND CENTRAL REVIEW

8.1 Central tissue collection
Microscope slides from formalin-fixed, paraffin-embedded tissue blocks from the placenta will be collected for central histology review (subject to availability of a sample and after local clinical pathology procedures have been completed). A copy of the clinical pathology report (de-identified) will also be obtained.

8.2 Central blood collection
Up to 5 plasma samples (for pharmacokinetic analysis) and 4 sets of dried blood spots on filter paper cards (for biomarker analysis) will be collected, according to the schedule of assessments. Total sample volume will not exceed 3.0 mL. Refer to the Biological Sampling Handbook for collection and processing procedures.

Any residual samples will be stored and used for future unspecified research.

8.3 Central imaging collection
MRIs and aEEG performed during hospital stay and video for General Movements assessment at 12 weeks of age will be submitted to the CTC so that these can be centrally reviewed. Refer to the Study Manual for scanning and collection procedures.

8.4 Central review

8.4.1 MRI central review
A panel of expert reviewers will be convened, and MRI scans will be reviewed centrally, in batches. The reviewers will be informed of the day of age and gestation, but will be blinded to the study treatment allocation and outcome of the baby. Images and spectroscopy results will be classified by severity and results compared between study treatment groups. MRI abnormalities caused by HIE evolve, particularly over the first days to weeks after birth. It is acknowledged that MRI scans will be done at varying times depending on site routine practice, condition of the baby, and availability of scan appointments. Interpretation will therefore take into account postnatal age and gestation at time of the scan. Some babies will be scanned before completion of study treatment, and even for those scanned after the 7 days of study treatment, the MRI appearances (and any potential benefits of study treatment) will not yet have fully evolved. However, if Epo is highly beneficial, severity of MRI abnormalities may still be reduced in the Epo treated group.

8.4.2 aEEG central review
A panel of expert reviewers will be convened, and aEEG files will be reviewed centrally, in batches. Reviewers will be informed of the gestation of the baby, but will be blinded to study treatment allocation and outcome of the baby. An aEEG will have generally been performed before and during study treatment, and will generally have been ceased before completion of study treatment. However, central review of aEEG recordings will allow the investigators to discern whether there are any effects of the early treatment doses on aEEG background or on seizure frequency/severity.

8.4.3 Placental pathology central review
The PAEAN study provides an opportunity for a comprehensive analysis of placental pathological abnormalities in babies with HIE. Pathologist reports will be collected and used to tabulate macroscopic abnormalities and results of any additional test results e.g. cytogenetics or microbiology. Microscope slides will be collected for central review, in batches, with the reviewing pathologist(s) blinded to the study group allocation. For microscopic abnormalities, central review will be regarded as primary.
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9 SAFETY REPORTING

9.1 Definitions

An ADVERSE EVENT (AE) is any untoward medical occurrence in a patient or clinical investigational subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not considered related to the medicinal product (see below).

Adverse events include the following:

- All suspected adverse drug or device reactions
- All reactions from drug or device – overdose, abuse, withdrawal, sensitivity, toxicity or failure of expected pharmacological action (if appropriate)
- Apparently unrelated illnesses, including the worsening (severity, frequency) of pre-existing illnesses
- Injury or accidents.
- Abnormalities in physiological testing or physical examination that require clinical intervention or further investigation (beyond ordering a repeat examination)
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a laboratory test).
- Any untoward event that occurs after the protocol-specified reporting period which the Investigator believes may be related to the drug or device.

Section 9.1.1 defines the AEs of interest for this study. Other AEs are not required to be reported unless they meet SAE, SUSAR and/or Outcome criteria.

A SERIOUS ADVERSE EVENT (SAE) is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (i.e. the subject is at risk of death at the time of the event),
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly/birth defect,
- other important medical events which, in the opinion of the investigator, are likely to become serious if untreated, or as defined in the protocol

NOTES:

(i) The term “life-threatening” in the definition of “serious” refers to an event in which the patient 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.

(ii) Important medical events which may not be immediately life-threatening or result in death or hospitalisation but which may jeopardise the patient or may require intervention to prevent one of the listed outcomes in the definition above should also be considered serious.

A SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR) is an SAE that is related to the drug or device and is unexpected (i.e. not listed in the investigator brochure or approved Product Information; or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the Subject Information Sheet and

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Informed Consent Form or elsewhere in the protocol. (FDA, Safety Reporting Requirements for INDs and BA/BE Studies, draft guidance, September 2010).

An event is causally related if there is a reasonable possibility that the drug caused the AE, i.e. there is evidence to suggest a causal relationship between the drug and the event (FDA, Safety Reporting Requirements for INDs and BA/BE Studies, draft guidance, September 2010).

9.1.1 Reporting to the central coordinating centre

The following adverse events of interest should be reported in the eCRF:

- Polycythemia requiring treatment (phlebotomy or dilutional exchange transfusion)
- New thrombosis of a major vessel > 7 days after receiving final dose of study drug
- AEs believed to be treatment-related resulting in treatment interruption or discontinuation

The following events should be reported as SAEs, regardless of whether they meet SAE criteria:

- Death
- Severe cardiorespiratory decompensation requiring CPR within 2 hours after receiving study medication
- New thrombosis of a major vessel ≤ 7 days after receiving final dose of study drug
- Any suspected case of Anti-Erythropoietin Antibody-Mediated Pure Red Cell Aplasia

For the purposes of this study, the following adverse events are not reported as SAEs:

- Frequently recognised complications or co-morbidities of moderate or severe hypoxic – ischaemic encephalopathy as follows:
  - Seizures
  - Oliguria/anuria/renal failure
  - Liver function abnormalities/jaundice (unless deemed to require exchange transfusion)
  - Respiratory failure or apnoea leading to need for mechanical ventilation (conventional or high frequency) or nitric oxide treatment
  - Hypotension/need for inotrope(s) or volume expansion
  - Thrombocytopenia and/or coagulopathy with or without need for red cell, plasma, platelet or other clotting factor therapy
  - Intracranial, subgaleal or other bleeding
  - Sepsis

Please note that seizures and the need for respiratory support will be recorded under the relevant neurodevelopmental outcome measures.

- Congenital anomalies or birth defects

9.2 Reporting of Serious Adverse Events (including SUSARs)

The investigator is responsible for reporting all Serious Adverse Events (including SUSARs) occurring during the study to the NHMRC Clinical Trials Centre within 1 working day of the investigator becoming aware of the event using the SAE form. SAEs must be reported up to 30 days from the end of study intervention.

SAE reports should be reported to the CTC as per the procedure documented in the Study Manual. The following information will be recorded for each Serious Adverse Event:

- Event description including classification
- Primary (and secondary if appropriate) diagnoses of event (if death/hospitalisation)
- Severity / Worst Grade
- Attribution to study intervention
- Expectedness (listed in product information)
- Action taken with study intervention, including rechallenge (if done)
- Outcome of SAE including end date if recovered
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9.2.1 Reporting to regulatory bodies
The CTC will provide SUSAR reports and SAE line listings to the Lead HREC and to Investigators for submission to Human Research Ethics Committees (HRECs) as required.
The CTC will submit ‘reportable safety events’ to the TGA in Australia and to the lead site in NZ for reporting to Medsafe.

10 TREATMENT INFORMATION

10.1 Erythropoietin
Epoetin alfa is recombinant human erythropoietin and is indistinguishable from Epo on the basis of functional assays. Epoetin alfa injection is a clear, colourless, sterile, preservative-free buffered protein solution available in pre-filled syringes and in vials.
Participating institutions will be responsible for preparing blinded study medication according to details in the Study Manual.

10.1.1 Supply of investigational product
Each participating site pharmacy will be responsible for ordering and maintaining a supply of study drug sufficient to treat 2-4 participants (depending on expected recruitment rate) each with 5 doses per participant. The study sponsor will supply the epoetin alfa used for a study participant in the form of blinded study kits, to be allocated at the time of randomisation.

10.1.2 Preparation and administration of blinded study treatment
Once a participant is randomised, the attending nurse will be responsible for drawing up and dispensing syringes of erythropoietin or an equivalent volume of 0.9% sodium chloride (without preservative) into syringes identified with the participant’s identifying details, study participant number and “PAEAN study drug, [concentration]” per local guidelines.

At the time of administration, the study drug/placebo will be added to an appropriate volume of 0.9% sodium chloride. The dose will be administered on a syringe pump and a low-dead space intravenous extension set via a peripheral intravenous, central venous or umbilical venous line at 1000 IU/kg birth weight (capped at 4000 IU) with the dose given over five (5) minutes. Care should be taken to flush the line with 0.9% sodium chloride after completion of the infusion to ensure administration of the complete dose.

10.2 Drug accountability
The Pharmacy Department at participating institutions will maintain a record of study drug dispensed for each participant.
11 STATISTICAL CONSIDERATIONS

11.1 Sample size
A sample size of 150 per treatment group is large enough to detect a 19% absolute risk reduction in the combined endpoint of death or severe/moderate motor/cognitive deficit assuming a control event rate of 46% (2), (decrease from 46% to 27%) and allowing for a 10% non-compliance/lost to follow-up rate with 90% power and a two-sided Type I error of 0.05. This estimated absolute risk reduction of 19% in adverse outcome is comparable to that seen in pilot studies (49). Such an effect size, if confirmed is clinically important.

11.2 Statistical analysis
Analyses of the primary and secondary outcomes will adhere to the Intention to Treat (ITT) principle, where all neonates randomised will be included. Analysis of safety endpoints will be according to treatment received, including only neonates who received at least one dose of treatment. All p-values will be two tailed without adjustment. A nominal significance level of 0.05 will be applied.

The proportion of neonates who have death or severe/moderate motor/cognitive deficit at 2 years will be compared using a chi-squared test. Continuous outcomes will be compared using t-tests where appropriate. Time-to-event outcomes will be displayed using Kaplan-Meier curves and comparisons where appropriate using the log-rank test.

Multivariable comparisons using regression methods will be used to explore the impact of key prognostic variables on outcomes.

11.3 Interim Analysis
A group sequential statistical approach will be used to perform a number of equally spaced interim analyses to assess accumulated safety data based on 25%, 50% and 75% of planned recruitment with 30 day safety data. A criterion for early stopping for reported SAEs at these interim analyses will use the Haybittle-Peto rule of 3 SDs (2p=0.003). In addition an asymmetric boundary will be used for interim analyses for mortality: stopping for benefit will be based on Haybittle-Peto rule of 3 SDs (2p=0.003), while stopping for harm will use a less conservative 2-sided alpha level of 0.01 at the first look and 0.05 at the second and third looks.
12 STUDY ORGANISATION AND COMMITTEES

12.1 Study coordination
Study coordination, monitoring, data acquisition and management and statistical analysis will be performed by the NHMRC Clinical Trials Centre.

12.2 Trial management committee
The NHMRC Clinical Trials Centre, in conjunction with the Principal Investigators will appoint a Trial Management Committee (TMC). A Trial Executive Committee (TEC) will be selected from the TMC in order to expedite decision-making and will be led by the Study Chair.

The TMC responsibilities include protocol development, study planning, monitoring of progress and participant safety, review of information from related research and implementation of recommendations from other study committees and external bodies (e.g. HRECs), and publications. The TMC will be responsible for selection and support of local investigators. The TMC will also monitor rate of recruitment and endpoint occurrence and will advise the ISDMC of variations.

The TEC is a subset of the TMC which meets more regularly on key scientific and/or operational issues impacting on study conduct. The TEC will meet at least once every quarter (or as the stage of the trial dictates) as the study progresses.

Members of the TMC will be required to attend regular meetings/teleconferences and assist in the promotion of the study. The trial executive will meet more frequently to expedite resolution of operational issues that do not require full TMC consideration. The TEC will be constituted under the terms of reference of the TMC.

The TMC will consider recommendations from the ISDMC about whether to continue the study as planned, modify, or stop it, based on safety analyses or other information.

12.3 Independent Safety and Data Monitoring Committee
This study will have an ISDMC. The ISDMC will be independent of the study and blinded to treatment arm, unless review of non-blinded data is required.

The ISDMC will provide independent assessment of participant safety and trial progress, making recommendations to the TMC about the continuation of the trial based on data made available by the unblinded statistician.

Agreed terms of reference for the ISDMC will be developed with the TMC.

A safety report for outcomes up to 30 days after last treatment will be provided to the chair of the ISDMC after 75, 150 and 225 babies have been enrolled.

12.4 Outcome Assessment Committee(s)
The Outcomes Assessment Committee (OAC) will provide central assessment of the primary outcome of disability. The OAC will also determine whether any information can be used to impute a primary outcome for participants who do not return for assessment of the primary outcome at 2 years of age. Examples of such information may include; results of assessment by a local paediatrician, evidence of severe cerebral palsy or developmental delay at an earlier visit, information obtained by telephone follow-up rather than an in-person visit, or otherwise partial data. The OAC, who will be blinded to the treatment assignment of the participant, will:

- receive all available data on the long-term outcome of these participants
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- assess whether they can definitively assign a primary outcome level and what it is, or
- if they do not have sufficient information to make that determination, determine if the value is missing.

The TMC will designate which committees or sub committees will analyse data such as aEEGs, MRIs, general movement analysis, biomarkers and placental pathology. The OAC will recommend and review procedures and protocols for the collection of review materials; perform review throughout the study according to the protocol; and document and report blinded outcomes of central review to the TMC.

13 ADMINISTRATIVE ASPECTS

13.1 Ethics and regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2007), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2013. To this end, no participant will be recruited to the study until all the necessary approvals have been obtained and the participant has provided written informed consent. Further, the investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a participant. In this circumstance the CTC, principal investigator and HREC must be advised immediately.

13.2 Confidentiality

The study will be conducted in accordance with applicable Privacy Acts and Regulations. All data generated in this study will remain confidential. All information will be stored securely at the NHMRC Clinical Trials Centre, University of Sydney and will only be available to people directly involved with the study and who have signed a Confidentiality Agreement.

Personal data identifying trial participants will be held securely at the CTC for the purpose of follow up if the participant is unable to continue/wishes to discontinue clinic based follow-up. The vital status of the participant (alive or dead) will be followed up through the Australian Institute of Health and Welfare (AIHW) National Death Index (NDI).

13.3 Protocol amendments

Changes and amendments to the protocol can only be made by the Trial Management Committee. Approval of amendments by the Institutional HREC is required prior to their implementation. In some instances, an amendment may require a change to a consent form. The Investigator must receive approval/advice of the revised consent form prior to implementation of the change. In addition, changes to the data collected, if required, will be incorporated in the amendment.

The investigator should not implement any changes to, or deviations from, the protocol except where necessary to eliminate immediate hazard(s) to trial participant(s).

13.4 Data handling and record keeping

All trial data required for the monitoring and analysis of the study will be recorded on the (e)CRFs provided. All required data entry fields must be completed. Data corrections will be made according to the instructions provided. The investigator will be asked to confirm the accuracy of completed CRFs by signing key CRFs as indicated.

Source documents pertaining to the trial must be maintained by investigational sites. Source documents may include a participant's medical records, hospital charts, clinic charts, the
investigator's participant study files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms. The investigator's copy of the case report forms serves as part of the investigator's record of a participant's study-related data.

The following information should be entered into the subject's medical record:

a. Participant’s name, contact information and protocol identification.
b. The date that the participant entered the study, and participant study number.
c. A statement that informed consent was obtained (including the date).
d. Relevant medical history
e. Dates of all participant visits and results of key trial parameters.
f. Occurrence and status of any adverse events.
g. The date the participant exited the study, and a notation as to whether the participant completed the study or reason for discontinuation.

All study-related documentation at ANZ sites will be maintained for 23 years following completion of the study.

**13.5 Study monitoring**

Data from this study will be monitored by Clinical Trials Program staff from the NHMRC Clinical Trials Centre (CTC) or their delegates. Monitoring will include centralised review of CRFs and other study documents for protocol compliance, data accuracy and completeness. Monitoring may include monitoring visits to investigational sites during the study for source data verification, review of the investigator's site file and drug handling records. The CTC will be given direct access to source documents, CRFs and other study-related documents. By signing the informed consent form, the participant gives authorised CTC staff direct access to their medical records and the study data.

**13.6 Audit and inspection**

This study may be subject to audit or inspection by representatives of the CTC, TMC or representatives of regulatory bodies (e.g. Therapeutic Goods Administration (TGA)).

**13.7 Clinical study report**

A Clinical Study Report which summarises and interprets all the pertinent study data collected will be issued which may form the basis of a manuscript intended for publication. The Clinical Study Report or summary thereof will be provided to the Human Research Ethics Committee(s) that approved the study and the funding agency (NHMRC).

**13.8 Publication policy**

The Trial Management Committee will appoint a Writing Committee to draft manuscript(s) based on the trial data. Manuscript(s) will be submitted to peer-reviewed journal(s). The first publication will be the report of the full trial results based on the main protocol using the study group name, with subsequent publications of data subsets in individual names based on contribution. The Writing Committee will develop a publication plan, including authorship, target journals and expected dates of publication. All publications must receive prior written approval from the TMC prior to submission.
REFERENCES

PAEAN – Erythropoietin for hypoxic ischaemic encephalopathy


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Appendix 1: Modified Sarnat Criteria

<table>
<thead>
<tr>
<th>Severity:</th>
<th>None or Mild encephalopathy</th>
<th>Moderate encephalopathy</th>
<th>Severe encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of consciousness</td>
<td>Normal or hyperalert</td>
<td>Decreased = reduced response to non-painful stimulation (&quot;lethargic&quot;);</td>
<td>Absent = only responds to painful stimuli (&quot;Stupor&quot;); or No or minimal response to pain (&quot;coma&quot;);</td>
</tr>
<tr>
<td>Spontaneous activity</td>
<td>Normal or increased</td>
<td>Decreased</td>
<td>None</td>
</tr>
<tr>
<td>Tone</td>
<td>Normal or increased</td>
<td>hypotonia = reduced trunk OR extremity tone OR both</td>
<td>flaccid = no tone</td>
</tr>
<tr>
<td>Suck reflex</td>
<td>Normal</td>
<td>Incomplete</td>
<td>Absent</td>
</tr>
<tr>
<td>Moro reflex</td>
<td>Strong and low threshold</td>
<td>Incomplete</td>
<td>Absent</td>
</tr>
<tr>
<td>Respiratory abnormality</td>
<td>Normal respiratory effort</td>
<td>Periodic breathing</td>
<td>Apnoea</td>
</tr>
</tbody>
</table>

Please note not all items may be assessable.

To qualify for the study, the baby must meet criteria in either the “moderate encephalopathy” or the “severe encephalopathy” columns on at least one examination conducted between 1 and 6 hours after birth:

- 3 out of 6 modified Sarnat criteria indicating moderate/severe encephalopathy

OR

- 2 out of 6 modified Sarnat criteria between 1 and 6 hours after birth, plus seizure(s) requiring anticonvulsant treatment (diagnosed either clinically or using EEG monitoring)

Since randomisation is also stratified for severity of encephalopathy, eligible babies should be classified as “moderate” or “severe” according to the column in which they meet the most criteria. In the event of a tie between moderate and severe criteria, if level of consciousness is severe assign the level of encephalopathy as severe otherwise assign as moderate.

Appendix 2: Head circumference


<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>3rd centile for head circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td>35</td>
<td>29.5</td>
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<tr>
<td>36</td>
<td>30.3</td>
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<td>32.9</td>
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</table>