PROTOCOL TITLE:

Mild Encephalopathy in the Newborn treated with Darbepoetin (MEND)

PRINCIPAL INVESTIGATOR:

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REGULATORY FRAMEWORK:

Please indicate all that apply:

☐	DOD (Department of Defense)
☐	DOE (Department of Energy)
☐	DOJ (Department of Justice)
☐	ED (Department of Education)
☐	EPA (Environmental Protection Agency)
☒	FDA (Food and Drug Administration)
☐	HHS (Department of Health and Human Services)
☐	Other:

Is this a clinical trial under ICH-GCP E6? ☒ Yes ☐ No

If yes, please confirm that the research team is familiar with and agrees to comply with the investigator requirements cited in ICH-GCP E6. ☒ Yes ☐ No

ICH-GCP E6 can be accessed by copying and pasting this URL into your browser: http://www.fda.gov/downloads/Drugs/Guidances/ucm073122.pdf

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1. Objectives

Primary Hypothesis: Darbe will improve neurodevelopmental outcomes in infants ≥34 week gestational age with mild encephalopathy who do not qualify for therapeutic hypothermia

Specific Aims:

- To assess the feasibility of administering one dose of Darbe in late pre-term (≥34 week) and term infants with mild encephalopathy, who do not qualify for TH
- Establish population pharmacokinetics of Darbe in late pre-term (≥34 week) and term infants with mild encephalopathy, who do not qualify TH
- To determine if Darbe improves neurodevelopemental outcomes in late pre-term (≥34 week) and term infants who do not qualify for TH

2. Background

Therapeutic hypothermia (TH) has become the standard of care for newborns diagnosed with moderate to severe neonatal encephalopathy (NE) at <6 hours of age. However, in current practice TH is not indicated in newborns who are NOT classified as moderate to severe NE on a <6 hours standardized neurological examination. Although perinatal acidemia is the initial screening component in the decision to treat the newborn with TH, the majority of newborns with perinatal acidemia do not receive TH because their neurologic examination is not abnormal enough to be classified as having moderate or severe NE. A retrospective review of infants found that as many as 20% of newborns who do not do qualify for TH encephalopathy will have abnormal short-term outcomes such as seizures, death from progressive asphyxia insult, abnormal brain MRI consistent with NE, abnormal neurologic examination at discharge, gastrostomy tube feeding, or feeding difficulties in the NICU. Preliminary data from a multicenter, prospective study (PRIME study) show 36% of infants with mild HIE had either abnormal neurological exam, aEEG or MRI; only 2% (1infant) had abnormalities in all 3 parameters. There are currently no therapies offered to infants with mild NE. Although, outcomes are thought to be better in infants with mild NE there is still a significant risk of neuronal injury in infants born with perinatal/fetal acidemia who are diagnosed with mild encephalopathy. Neuroprotective strategies aimed at improving early childhood outcomes are needed. Erythropoiesis stimulating agents (ESA) have been shown to provide neuroprotection, improving short and long-term neurologic outcome in brain injury and NE in neonates. ESA may work through several important mechanisms including reduced inflammation, limited oxidative stress, decreased apoptosis and white matter injury, as well as via pro-angiogenic and neurogenic properties. Darbepoetin alfa (Darbe), a recombinant human erythropoietin (EPO)–derived molecule, has an extended circulating half-life and comparable biological activity to EPO. The proposed study is a Phase II trial of early Darbe administered to infants with mild NE. The long-term objective of the proposed research is to decrease the risk of long-term disabilities in infants with mild NE who do not qualify for hypothermia therapy.

Preclinical Trials

In recent years, ESA have been studied extensively in preclinical trials of neuroprotection prompted by the finding that EPO receptor is expressed in the human brain (in cultured neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells)(1-3). Importantly, both EPO and Darbe administered peripherally can cross the blood-brain barrier by way of extracellular pathways in amounts that can account for their neuroprotective actions.(4) Animal models have shown EPO to activate cellular mechanisms that promote cellular maturation, inhibition of apoptosis, neurovascular remodeling, revascularization and neurogenesis. (5-9) Exogenously administered EPO also enhances endothelial progenitor cell mobilization from the bone marrow, amplifies the production of neural progenitor cells, and stimulates oligodendrogenesis. (10-12)

With respect to the treatment of ischemic brain injury in experimental animal models of perinatal brain injury, systemic administration of EPO improves functional and histological recovery. EPO reduced infarct volume and improved functional and neurobehavioral performance when given immediately prior to or after neonatal brain injury using the Vannucci-Rice model for neonatal HI (13-16). Neurobehavioral testing revealed significant enhancement of muscle strength, limb placing reflexes, motor coordination and neurosensory skills. Recently, Traudt et al showed, in a non-human primate model of HIE, that EPO combined with hypothermia decreased death and moderate/severe cerebral palsy in comparison to placebo or hypothermia only. Additionally, neuroimaging studies were improved with combined EPO/hypothermia treatment. (17)

Similarly, Darbe administration following cortical impact injury in adult rats improved cerebrovascular function and reduced histological damage in a dose and time dependent manner(18). Weekly administration of Darbe conferred histological and behavioral neuroprotection after intracerebral hemorrhage in rats similar to that of EPO administration.(7, 19, 20)

Finally, following focal cerebral ischemia in rats (middle cerebral artery suture-occlusion), Darbe-treated rats showed decreased infarct volume and total infarct areas as well as improved neurologic scores relative to vehicle-treated animals. (20)

In summary, there is convincing data in a variety of animal models that ESA can cross the blood-brain barrier and enhance neurological recovery following stroke, NE, and trauma. This is accomplished through several important mechanisms such as inhibition of apoptosis and enhanced neurogenesis.

Previous Human Experience

Adult Clinical Trials

ESA use in critically ill adult patients

In recent years, EPO clinical trials have been designed to assess neuroprotection in a variety of conditions including stroke, subarachnoid hemorrhage, out-of-hospital cardiac arrest, and cardiac surgery(21-26). In these settings, the dose of EPO ranged from 30,000 U/dose X3 doses every 48 hours(26); 40,000 IU of IV EPO within 6hrs of symptoms, at 24h and at 48h(22); 40,000U IV q12h for the first 48h after ICU admission(23); or 375-1500 U/Kg x 3 daily doses(24).

The overall outcomes have been quite variable, ranging from favorable to no difference in the outcomes measured between treatment groups. Among favorable outcomes, Tseng observed that in those patients <60 years old and non-septic, the EPO group developed significantly less cerebral vasospasm, impairment in cerebral autoregulation and neurological deficits (as measured by the National Institutes of Health Stroke Scale)(26). Haljan et al. observed a trend in reduction of neurocognitive dysfunction in patients undergoing coronary artery bypass surgery who received increasing doses of EPO divided in 3 daily doses, starting the day before surgery(24). In a recent retrospective matched case control study of EPO and Darbe, Talving et al observed that ESA administration following severe traumatic brain injury in adult patients was associated with a significantly improved in-hospital survival rate without increase in morbidities in comparison to matched controls(25).

In some, but not all of these studies, EPO administration was associated with a higher rate of death, intracranial hemorrhage, thromboembolic events and brain edema (22) (23). These results are in contrast to a smaller trial in critically ill patients that were also treated with EPO but who **did not receive thrombolytic therapy**, as was the case in patients with the complications described above(21).

A 2016 analysis of ESAs in critically Ill trauma patients found a significant improvement in mortality without an increase in the rate of proximal deep vein thrombosis(27).
In conclusion, further studies of the potential therapeutic effects of ESA are warranted in select populations, such as newborns with hypoxic-ischemic injury at birth, with close monitoring of outcomes and adverse effects.

Studies of ESA in Premature Infants

The administration of human recombinant EPO in preterm infants has been studied as an alternative to red blood cell transfusion in the treatment of anemia since the late 1980s. Between 1991 and 2009, 2,723 preterm infants were enrolled in 33 randomized controlled trials to evaluate the safety and efficacy of EPO as a treatment for anemia of prematurity. Treatment regimens varied widely, ranging from 70 to 5000 U/kg/week, with duration of therapy ranging from 2 weeks to several months. Halperin et al reported data from the first pilot study on the treatment of anemia of prematurity in 1990(28). Since

then, a multitude of studies have been performed examining a variety of EPO doses from low <500 U/kg/week to high >500 U/kg/week and treatment periods from early (started before 8 days of age) versus late (between 8 - 28 days after birth).(29-31). The NICHD NRN study of early erythropoietin therapy for anemia of prematurity examined the effects of EPO treatment (400 U/kg 3 X/ week) initiated by 4 days after birth and continued through 35 weeks postmenstrual age in preterm infants < 1250g birth weight. This study revealed no impact in transfusion requirement, but was important in that it demonstrated no increase in adverse events with similar rates of hospital morbidities, mortality, length of hospital stay, neutropenia, hypertension and seizures in the EPO treated and placebo/control infants. Follow-up at 18-22 months' corrected age revealed similar rates of neurodevelopmental impairment and need for rehospitalization between groups(32). Gumy-Pause et al studied higher doses (up to 5000 U/kg/week) of EPO and once again demonstrated no impact on episodes of infection, NEC, sepsis and neutropenia between low (1250 U/kg/wk) and "high-dose" (up to 5000U/kg/wk) EPO(33). In a small ancillary study to the NRN trial, 16 premature infants were treated with EPO or placebo for anemia of prematurity, and **Bierer et al reported an association between higher EPO levels (> 500 mU/mL) and higher Mental Developmental Index scores** on the Bayley Scales of Infant Development II at 18-22 months' corrected age(34). Similarly, **Brown et al** reported improved neurodevelopmental outcomes in a secondary analysis of 82 preterm infants born <1500g and < 30 weeks' gestational age treated with erythropoietin for anemia. **Increasing cumulative EPO exposure was associated with higher BSID II Mental Developmental Index scores (35). N**eubauer et al **reported a similar beneficial effect on 10-13y outcome of 148 preterm infants treated with EPO** under a variety of dosing regimens: the EPO group scored significantly better than untreated children in the overall developmental assessment (55% vs. 39% normally developed, $p<0.05$) as well as in the psychological examination (mean composite HAWIK-III IQ score, 90.8 vs. 81.3, $p < 0.005$)(36). **Over this wide range of dosing and duration of therapy, EPO and Darbe has been quite safe in neonatal populations. No study of EPO treated newborns has reported an increase in thrombotic complications similar to those observed in adults.**

Several recent single-center trials reported on the **safety and pharmacokinetics of "neuroprotective" doses of erythropoietin in premature infants** using doses that are approximately 10 fold higher than those used for anemia of prematurity. Juul and colleagues performed a prospective, open-label dose-escalation trial in which 30 infants were treated with high dose recombinant human erythropoietin (Epoetin Alfa Recombinant) and compared with 30 concurrent control infants(37). Infants received doses of 500U/kg/dose, 1000U/kg/dose or 2500U/kg/dose for three doses at 24 hour intervals starting on day 1, with ten infants in each group. The researchers found that both the 1000U/kg/dose and 2500U/kg/dose produced peak serum EPO concentrations that were analogous to neuroprotective concentrations seen in animal and adult studies. High dose EPO followed nonlinear pharmacokinetics as decreased clearance was observed with the highest dosing regimens. No increase in adverse events was reported for the EPO treated infants when compared with the control infants(37).

Fauchère and colleagues performed a randomized, double-masked trial in which preterm infants between 24 and 31 weeks GA were given recombinant human EPO (Epoietin Beta in doses of 3000U/kg birth weight) or placebo at 3, 12-18, and 36-42 hours after birth. A multicenter trial looked at preterm infants (26-32 weeks gestation) and found no improvement in neurodevelopmental outcome at 2 years corrected age, and no adverse effects using rhEPO 3000 U/kg.
Once again, **administration of high dose EPO resulted in no significant adverse events(38)**.

Regarding the administration of Darbe in preterm infants, Warwood el al showed that a single dose of Darbe accelerated effective erythropoiesis in a pilot study of 12 preterm infants(39). The same group of investigators reported that IV administration to neonates resulted in a shorter half-life, a larger volume of distribution and more rapid clearance(40).

Ohls et al. performed a **randomized, placebo controlled study** to assess the safety and efficacy of **Darbe administered to preterm infants** (500-1250 grams birth weight). In this study they reported preterm infants who received ESAs (Darbe 10μg/kg or EPO 400 U/kg) had significantly better cognitive outcomes at 18-22 months and at preschool testing compared with placebo recipients, with no adverse events reported.(41)
Natalucci et al. recently published a study using prophylactic early high-dose recombinant human erythropoietin in preterm infants (26-31 weeks gestation). 228 participants were randomized to receive either erythropoietin 3000 IU/kg and 220 received the placebo. Erythropoietin was given IV at 3 hours, 12-18 hours, and at 36-42 hours after birth. They found that among infants who received prophylactic erythropoietin there was no significant difference in neurodevelopmental outcome between the groups. No adverse events were reported(42).

Trials of EPO in term infants with NE

Recent studies have evaluated the use of EPO in **term infants with NE** not treated with hypothermia. The first was a randomized prospective study of 167 term infants with moderate/severe NE conducted by Zhu et al(43). Infants were randomly assigned to receive either EPO (N=83) or conventional treatment (N=84). EPO treated infants received either 300U/kg of rhEPO (N=52) or 500U/kg of rhEPO every other day for 2 weeks starting within 48 hours of birth. Study participants were followed to 18 months of age and underwent detailed neurodevelopmental assessment. Primary outcome was death or moderate/severe disability defined as CP, severe hearing loss, blindness, gross motor function classification levels 3 through 5, and a MDI <70 on the Bayley Scales of Infant Development II. Complete outcome data were available for 91.6% of participants. **EPO treated infants had significantly lower rates for death or moderate to severe disability** (24.6% [18/73] infants versus 43.8% [35/80]) in control infants; P=0.017). Subgroup analyses revealed benefit from EPO treatment only in babies with moderate NE. No differences in primary outcomes or side effect profiles were noted for the two studied EPO doses (43).

A study conducted by Elmahdy et al, was a pilot study that examined the safety and efficacy of much higher doses of EPO administration upon generation of nitric oxide (NO), a mediator of hypoxic injury. This was a case-control study with 3 groups of infants (15 infants per group). The first group was comprised of normal healthy term infants; the second was comprised of term infants with mild/moderate NE who were administered 2500U/kg of subcutaneous EPO daily for 5 days; and the third group was an NE control group that received no EPO. Baseline serum concentrations of NO were measured in the normal healthy neonates, and at baseline and 2 weeks for the two NE groups. In addition, the two NE groups underwent encephalography at baseline and 2-3 weeks and MRI at 3 weeks of age. Neurological assessment and neurodevelopmental screening (Denver Developmental Screening Test II) were performed at 6 months of age. Compared with the healthy term infants, the NE groups had higher serum NO levels. Though the two NE groups did not differ in severity of illness, NO levels, seizure burden or EEG background activity at baseline, the EPO treated group had statistically lower serum NO levels, fewer neurodevelopmental deficits and improved EEG background at follow-up. MRI findings did not differ between groups. No side effects related to EPO administration were identified despite close monitoring for allergic reactions, venous thrombosis, renal/hepatic effects, hypertension or electrolyte disturbances(44).

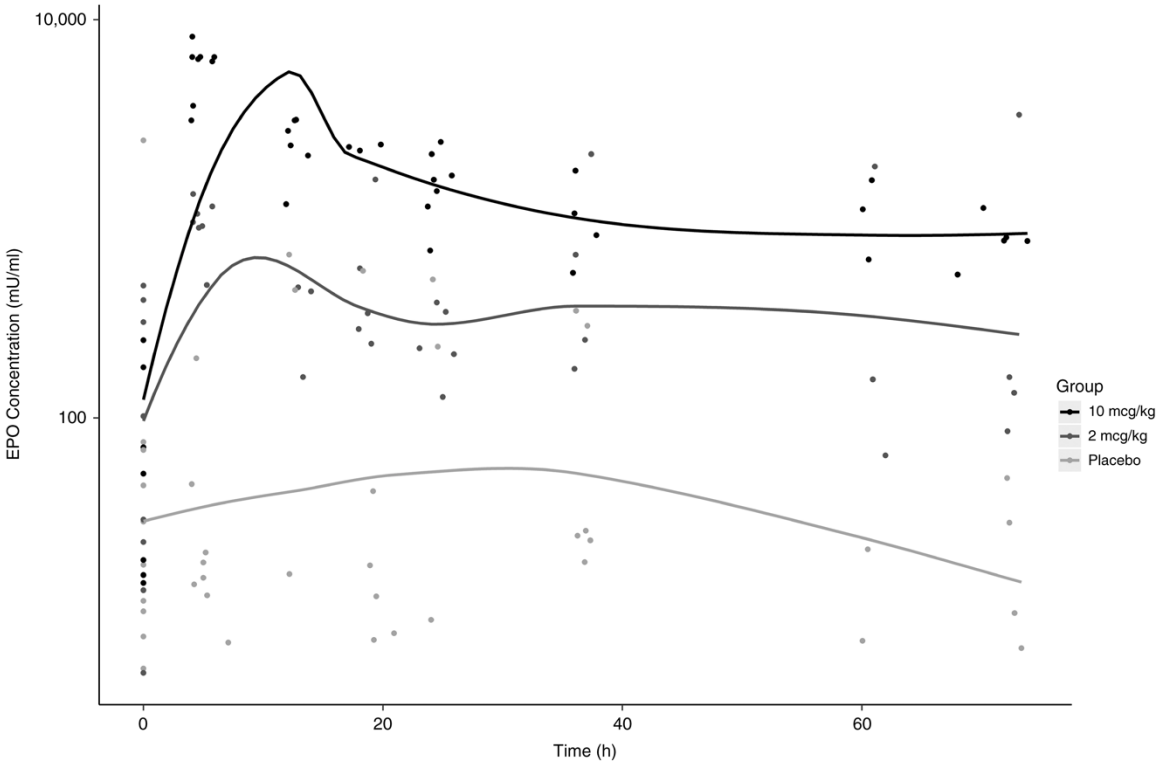
In 2012, an open label, dose-escalation, phase I study, enrolled 24 term infants undergoing TH for moderate/severe NE. Patients received 1 of 4 Epo doses intravenously: 250 (N=3), 500 (N=6), 1000 (N=7), or 2500 U/kg per dose (N=8). They gave up to 6 doses every 48 hours beginning at <24 hours of age. Epo did not follow linear pharmacokinetics, but excessive accumulation did not occur. At 500, 1000, and 2500 U/kg Epo, $t_{1/2}$ was 7.2, 15.0, and 18.7, and the area under the curve was 50,306, 131,054, and 328,002 U*h/L, respectively. They noted drug clearance at a given dose was slower than reported in uncooled preterm infants. No deaths or serious adverse events were seen. They found that Epo at 1000 U/kg given intravenously in conjunction with hypothermia is well tolerated and produces plasma concentrations that are neuroprotective in animals (45).

In 2016 a phase II double-blinded, placebo-controlled trial randomized newborns with moderate/severe NE to treatment with hypothermia alone or high-dose erythropoietin in addition to hypothermia. 50 infants were enrolled. They found that high-dose erythropoietin and hypothermia resulted in less MRI injury and improved neurodevelopmental outcomes at 12 months of age. There were no safety issues reported(45).

The therapeutic potential of EPO and its chemical analog Darbe for NE brain injury in the newborn appears to be safe.

Low Dose vs. High Dose Darbepoetin

In 2015 a multi-center study was published to assess safety and pharmacokinetics of Darbe in infants undergoing TH for NE. It evaluated 30 term infants with moderate to severe NE who were undergoing hypothermia therapy and randomized to standard of care TH or TH and Darbe low dose (2 μg/kg) or TH and Darbe high dose (10 μg/kg) given intravenously within 12h of birth and at 7 days. Adverse events were documented for 1 month and pharmacokinetics were evaluated. Adverse events were similar to placebo and historical controls. Following the first dose (hypothermia conditions) at 2 and 10 μg/kg, $t_{1/2}$ was 24 and 32 hours, and the area under the curve was 26,555 and 180,886 h*mU/ml*, respectively. The clearance of the drug was not noted to be different between the doses. At 7 days (normothermia conditions), $t_{1/2}$ was 26 and 35 hours, and the area under the curve was 10,790 and 56,233 h*mU/ml*, respectively(46).



This study showed that the **10** μg/kg dose produced a median AUC_{inf} of 180,886 h*mU/ml after the first dose, which is comparable to previous report of neuroprotection in animal studies. (47)

In summary, despite more than 20 years of use, few safety concerns have been identified with the use of ESAs in preterm and term infants. Furthermore, there is emerging evidence that early use of EPO or Darbe in extremely low birth weight infants can improve neurological outcomes. Additionally, recent research has given guidance as to the pharmokinetics of Darbe in neonates with moderate/severe encephalopathy.

3. Study Design

Eligible patients will be identified by the admitting neonatologist or by the research nurses based on admission diagnosis (perinatal depression, perinatal acidemia, HIE or NE, encephalopathy, clinical assessment for the need for therapeutic hypothermia).

Inclusion Criteria: Infants will be eligible for the MEND trial if they have a gestational age ≥ 34 weeks by best obstetric estimate, are <24 hours old and have evidence of mild encephalopathy and **DO NOT** have moderate-severe acute NE as defined by Shankaran et al based on a modified Sarnat examination performed at <6 hours of age (48).
Eligibility will include criteria presently used in the NICU to initiate therapeutic hypothermia and used in the NICHD NRN hypothermia trial:

1) History of an acute perinatal event (abruption, cord prolapsed, severe fetal heart rate abnormality, or meconium staining)
2) Infant is evaluated for hypothermia therapy and **DOES NOT** meet clinical criteria for TH.
3) Infant has an IV for clinical treatment

Exclusion Criteria

1) Moderate/Severe encephalopathy on modified Sarnat examination at < 6 hours of age
2) Major congenital and/or chromosomal abnormalities
3) Prenatal diagnosis of brain abnormality or hydrocephalus
4) Severe growth restriction (≤ 3% for gestational age)
5) Central venous hematocrit >65%, platelet count >600,000/dL, and/or neutropenia (ANC<500 µL)
6) ECMO
7) Infant judged critically ill and unlikely to benefit from neonatal intensive care by the attending neonatologist

After eligibility is confirmed, with approval from the Attending Care Provider, the parent or legal guardian will be approached for consent (as noted below under consent process). All patients eligible for this study will be managed in the NICU.

Drug administration: Each center will obtain their own Darbe through their research pharmacies. Randomization will take place at each site using a provided block-design randomization table created by UNM research pharmacy. The site pharmacy will then dispense the study drug to the research nurse in a closed container. Darbe and vehicle are the same in appearance, and will be labeled “study drug”.

DESIGN:

This is a Phase II placebo-controlled randomized, blinded, feasibility trial.
The multisite study will consist of 80 infants (n=40 in each arm) ≥34 week GA infants with NE who **DO NOT** qualify for hypothermia therapy, that will be randomized to receive either Darbe,

(10 µg/kg IV), or placebo. **We plan to enroll 20 participants at the University of New Mexico**. A total of 80 infants at all sites (see table below) will be enrolled.

Dr. DuPont (PI) Holds the IND for Darbepoetin and mild NE (IND 132207) and will remain as a study team member and overall PI of this multi-center study. Treatment will be randomized by the pharmacy and blinded to the caregivers. All groups will receive the dose within 24 hours of birth.

√		**Intervention**
Experimental groups Darbe	N=40	10 mcg/kg/dose Darbe IV <24 hours of age
Control group Placebo	N=40	Normal saline placebo dose IV at <24 hours of age

STUDY PROCEDURES:

Timeline for the study:

Anticipated time to recruit 80 patients will be 2 years. Centers that will participate in this Phase II study will include University of New Mexico Children's Hospital, University of Utah Medical Center, Intermountain Medical Center, Primary Children's Medical Center, The University of South Florida, Stanford University, and University of Texas Southwestern.

Once the infant meets eligibility criteria and consent has been obtained from the parent(s), the infant is a study participant.

Data collection

At study entry maternal demographics, specifically: age, education, race, ethnicity will be collected. A modified Sarnat exam will be repeated at discharge, transfer, or 96 hours of age (which ever occurs first) and documented. We will collect vital signs, MRI, hemoglobin/hematocrit, CBC with differential, liver function tests, and complete physical exam if they are done as indicated by clinical care. MRIs done for clinical indication will be de-identified and sent to a pediatric neuroradiologist for a centralized read. Once de-identified, these will be sent via CD or LifeImages to UT Southwestern where they will be read by the neuroradiologist. We are requesting waiver of consent to send de-identified MRIs to UT Southwestern for an additional reading. All MRIs have been and will be ordered per clinical indication and are not required under the study protocol.

Immunogenicity Testing

The vast majority of babies admitted to the NICU have a CBC with diff drawn on admission. If there is at least 0.5mL of blood remaining (scavenged blood) after analysis, we will collect that blood and use as our baseline sample for potential immunogenicity testing. If the baby remains in the NICU for 2 weeks and has a blood test done for clinical reasons, we will attempt to keep 0.5mL (scavenged blood). Additionally, a letter (attached) will be given to the PCP stating that the patient is

enrolled in a research study. If there are concerns for anemia and the PCP orders a CBC within the first year of life we will ask for 0.5mL of blood (scavenged blood). We will ask the lab to hold “scavenged” blood and store for up to 6 months beyond enrollment of the last patient.

PK Sampling

Blood samples will be used to establish population pharmacokinetics. Scavenged blood samples of 0.05 mL of blood will be collected from all clinical blood draws throughout the infant’s hospitalization and sent to the University of Iowa for population pharmacokinetics using meso-scale discovery assay to detect Epo levels. Since a baseline sample is also required for immunogenicity testing we will collect an extra 0.3 mL of blood with the “pre-drug” blood work drawn in the NICU. Study participants will have the option of opting in for this additional blood draw. This will ensure that there is enough blood for potential immunogenicity testing and pK analysis. Every effort will be made to obtain this sample with a blood draw that is needed for clinical purposes (such as a glucose check). All other pk samples will be off of scavenged blood. Parents will be given the option to opt into this part of the study on the consent form.

Parents of participants will be contacted by phone call at 4 months of age which will include developmental screening via a modified Ages and Stages questionnaire (attached), number of hospitalizations, seizures, need for assisted respiratory support, failure to thrive, medication use, use of early intervention services, hearing impairment, any concern with vision.

Participants will have neurodevelopmental testing (Bayley III and Gross Motor Function Assessment) performed at 8-12 months of age. We will also conduct a brief medical exam including weight, length, head circumference, blood pressure, and heart rate.

In addition, we may contact the child’s primary care provider and ask for the baby’s most recent medical history and the result of the most recent hematocrit.

Data will be kept for analysis for up to 10 years.

Standard of Care vs. Research-Related Procedures:

Research-Related procedures:

1. Study drug can be given as soon as the infant is found to **NOT** qualify for TH but before 24 hours of age.
2. Extra 0.3 mL of blood drawn prior to drug administration (optional)
3. Neurological examination at discharge, transfer, or 96 hours of age (which ever occurs first)
4. Follow up phone calls at 4 months of age
5. Bayley Scales of Infant Development examination at 8-12 months corrected age.
6.

7. Weight, length, head circumference, heart rate, and blood pressure at 8-12 months.
8. Medical records from primary care provider including hemoglobin and hematocrit if available when indicated.

Standard of Care:

1. Monitoring and routine supportive care.

Safety

For the purpose of this study, an **adverse event** (AE) will be defined as any adverse change from the patient's baseline condition that occurred following the administration of the study drug through the end of the study period. Safety will be evaluated during the first 7 days of life or until hospital discharge (whichever comes first) by documenting potential adverse events such as (but not limited to) alterations in blood pressure, secondary infections, neutropenia, thrombotic/vascular events, hematologic events (platelets, Hct level, polycythemia), and hepatic/renal function that are outside of normal range for the study population.

We will assess for the rare complication (1 in 10,000 in adults with renal failure) of pure red blood cell aplasia. The vast majority of babies admitted to the NICU have a CBC with diff drawn on admission. If there is at least 0.5mL of blood remaining (scavenged blood) after analysis, we will collect that blood and use as our baseline sample. Similarly, most babies admitted to the NICU have electrolytes or bilirubin levels drawn at 24-72 hours of age, if there is at least 0.5mL of blood remaining (scavenged blood) after analysis, we will collect that blood and use as our second sample. If the baby remains in the NICU for 2 weeks (uncommon) and has a blood test done for clinical reasons, we will attempt to keep 0.5mL (scavenged blood) to assess for pure blood cell aplasia. Additionally, a letter (attached) will be given to the PCP stating that the patient is enrolled in a research study. If there are concerns for anemia and the PCP orders a CBC within the first year of life we will ask for 0.5mL of blood (scavenged blood). We will ask the lab to hold "scavenged" blood and store for up to 6 months beyond enrollment of the last patient.

We will also ask for the medical records from the child's primary care provider and their most recent hematocrit/hemoglobin.

Long Term Safety will involve phone calls at 4 months of age which will include developmental screening via Ages and Stages questionnaire, hospitalizations, seizures, need for assisted respiratory support, failure to thrive, medication use, use of early intervention services, hearing impairment, any concern with vision. In addition we will contact the child's primary care provider and ask for the child's medical records and most recent hematocrit/hemoglobin to assess for safety.

Serious adverse events (SAE) will be defined as any event that results in: death, a life threatening event (major venous thrombosis, stroke, and/or severe hypertension), persistent or significant disability/ incapacity, and/or prolongs inpatient hospitalization.

SAEs will be reported until 30 days following final cessation of therapy or until hospital discharge, whichever comes first.

Evaluation	Screening	Pre-Entry (Prior to 24 hrs)	Entry (<24 hr of age)	discharge, transfer, or 96 hours of age (which ever occurs first)	3-10 days	Discharge	4 months corrected age	8-12 months corrected age
Screen admissions to NICU	X	X						
Documentation of Gestational Age		X						
Informed Consent		X						
Maternal Demographics and History			X			X		
Blood Pressure			X		X	X		X
Modified Sarnat Exam				X				
MRI as available from clinical care					X	X		
Hematology (CBC/transfusions/phlebotomy loss) as available from clinical care			X			X		X
Administration of study drug IV			X					
Liver/kidney Function Tests (as clinically available)					X			
Complete Physical Exam including weight, head circumference and length			X			X		
Telephone contact to review current status							X	
Ages and Stages phone questionnaire							X	
Phone/in person survey on hospitalizations, seizures, need for respiratory support, failure to thrive, medication use, use of early intervention, hearing impairment, concerns with vision							X	X
Neurodevelopmental assessment Bayley III & Standardized Neurologic exam								X
Weight, length, head circumference, blood Blood pressure, and heart rate								X

4. Prior Approvals

- Tara DuPont, MD holds the IND (IND 132207) for this trial.
- See the attached departmental review form.

Study Resources: Trained NICU research nurses are available at the University of New Mexico, the University of Utah, and Primary Children's Medical Center NICU's. All sites have a locked office with locking cabinets in which study data will be kept. All participating infants will be assigned a study number and identified for study purposes by that number only.

- All sites have the most current version of the protocol, consent document, and HIPAA authorization.
- We are seeking approval from the IRB's at each site, when obtained they will be added to this document as a modification.
- All modifications will be communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.
- All engaged participating sites will safeguard data as required by local information security policies as they are all actively involved in neonatal clinical trials.
- All local site investigators will conduct the study appropriately.
- *All non-compliance with the study protocol or applicable requirements will reported in accordance with local policy.*

Control of Investigational Devices/Drugs: The study drug will be stored in, controlled and dispensed by, the Investigational Research Pharmacy. Labeling for the study drug will be done per standard investigational drug guidelines employed by the research pharmacy

Dr. Tara DuPont, the sponsor-investigator, will maintain complete and accurate records showing any financial interest in 54.4(a)(3)(i), (a)(3)(ii), (a)(3)(iii), and (a)(3)(iv) of the CFR paid to clinical investigators by the sponsor of the covered study. Dr Tara DuPont, the sponsor-investigator, will also maintain complete and accurate records concerning all other financial interests of investigators subject to part 54 of the CFR.

Jean Lowe and qualified study team members will prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each participant who is given darbepoetin or employed as a control in the study. Jean Lowe and the Clinical Research manager, will be responsible for preparing and maintaining these case history reports at the University of New Mexico.

Jean Lowe or her designee will retain the records and reports required by 21 CFR 312.57 (c) for two years after a marketing application is approved for the drug; or if the application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

Jean Lowe will ensure that reserve samples and/or lot numbers of the study drug, darbe will be maintained at the University of New Mexico and released to the FDA upon request, in accordance with, and for the period specified in section 320.38. These study drug samples will be maintained in a -80 freezer at the University of New Mexico for later testing for pK analysis.

Assurance of IRB Review:
Jean Lowe and participating clinical investigators will promptly report to reviewing IRBs all unanticipated problems involving risk to human participants or others according to IRB policy. Jean Lowe and/or study staff will be responsible for preparing and submitting these reports to the University of New Mexico IRB and maintaining files of IRB approvals/acknowledgements.

5. Data Analysis

This will be a multicenter pilot/feasibility trial to assess Darbe for infants who do not qualify for TH. Data from this trial will be used to power a large multicenter RCT. Primary outcome variable will be the incidence of abnormal neurodevelopmental score by Bayley III <85 or GMFS of >1 at 8-12 months of age. Chi-square or Fisher exact statistical tests will be performed for all categorical values. For continuous variables, t tests will be performed. All outcomes will be analyzed based on intention to treat inclusion of all randomized patients. We estimate infants who received Darbe will experience an estimated 30% decrease in neurodevelopmental impairment, defined as BSID III cognitive score or motor score <85, or a Gross Motor Function Classification Scale score >1. A total of 40 infants per group will be required to identify a 30% difference between groups.

6. Provisions to Monitor the Data to Ensure the Safety of Subjects

The data and safety monitoring board (DSMB) is composed of members qualified by training and experience to monitor the progress of the investigation:

1) John Philips, MD Professor of Neurology, University of New Mexico. Dr. Philips will be the DSMB Chair for this project.
2) Kristi Watterberg, MD. Professor of Pediatrics, University of New Mexico. Dr. Watterberg has led and conducted multiple multicenter clinical studies .
3) Lauren Jantzie, PhD. Division of Neuroscience, Department of Pediatrics, University of New Mexico
4) Pablo J. Sanchez, MD. Professor of Pediatrics, Nationwide Children's Hospital, Columbus, Ohio.

Monitoring plan will include:

1) The DSMB will review the study data for non-compliance and participant safety (adverse events, unanticipated problems, etc.) after the first 20 patients have been enrolled and every 6 months until study completion

2) The DSMB will report their findings to the sponsor-investigator after each meeting
3) The sponsor-investigator will report DSMB findings to all participating clinical investigators after each meeting. Each participating investigator will submit the DSMB review to their IRB with corresponding documentation of the submission sent to Dr. Tara DuPont
4) All SAEs will be reported to Dr. DuPont within 24 hours of the site learning of the event. Each site PI will be responsible for reporting the serious or unexpected adverse events to their local IRB in accordance with the local IRB policies. Dr. DuPont will be responsible for notifying the DSMB chair within 24 hours of receiving the report and notifying the FDA in an IND safety report in accordance with FDA regulations. The DSMB will determine if the adverse event changes the risk to study subjects. If the information changes the known risk to subjects, the DSMB report of this event will be released to all participating investigators by Dr. DuPont.
5) Criteria for Withholding Study Drug: Neutropenia (ANC <500/μL), hematocrit of >65%, stroke, symptomatic clot, or hypertension (blood pressure 2 SD greater than the mean for age).

7. Withdrawal of Subjects

This is a single intervention study. If the infant is eligible and consented then the study drug will be given. After the delivery of the drug the intervention is complete. The family may choose to withdraw from the study at any time. Follow-up phone calls and neurodevelopmental testing will occur between 4-12 months of age.

8. Risks to Subjects

Darbe use in adults can increase the risk of abnormal blood clot formation, increase red blood cell number, decrease white blood cell number, increase blood pressure, serious cardiovascular risks, including stroke, venous thrombus, and death. Studies of Darbe use in adult cancer patients have shown increased cancer growth. To date there is no information that these drugs would increase any such complications in infants. There may also be side effects and discomforts that are not yet known.

The infant will be assigned to a treatment group by randomization. The treatment the infant receives might be less effective, not effective, or have more side effects than another study treatment.

9. Potential Benefits to Subjects

A potential benefit maybe improved neurodevelopmental outcome due to improved neuronal recovery from the administration of Darbe. In addition, subjects will receive close developmental follow-up via phone calls and exam at 8-12 months of age.

10. Recruitment Methods

Infants will be recruited for the study by the research nurses, investigator and co-investigators during hospitalization in the NICU at each medical center following protocol criteria.

11. Other Sites may include:

University of Utah Hospital
50 N Medical Drive
Salt Lake City, UT 84132

Intermountain Medical Center (Affiliated with University of Utah)
5121 Cottonwood Street
Murray, UT 84107

Primary Children's Hospital (Affiliated with University of Utah)
100 North Mario Capecchi Drive
Salt Lake City, UT 84113

UTSW Sites (DUA attached. This site will not be enrolling patients but will be sent MRIs of enrolled infants)
Lina Chalak, MD
University of Texas Southwestern Medical Center
5323 Harry Hines BLVD, Dallas, TX 75390-9063
Lina.Chalak@UTSouthwestern.edu

Parkland Hospital
5200 Harry Hines BLVD.
Dallas, TX 75235

Children's Health Dallas
1935 Medical District Dr.
Dallas, TX 75235

University of South Florida Site: (No data will be transferred until DUA has been obtained)
Jaime Flores-Torres, MD
5 Tampa General Cir, Suite HMT 450.19
Tampa, FL 33606-3601
jflorest@health.usf.edu

University of South Florida/ Tampa General Hospital
1 Tampa General Circle
1st floor TGH F170
Tampa, GL 33606

Stanford Site: (No data will be transferred until DUA has been obtained)

Krisa Van Meurs, MD
Stanford University/ Lucile Packard Children's Hospital
725 Welch Rd.
Palo Alto, CA 94304
vanmeurs@stanford.edu

Each site will be responsible for screening, recruitment, and consent process for study subjects. Data collected at each site will be de-identified prior to transferring to the University of New Mexico, where it will be analyzed on an intention to treat basis. All participating infants will be assigned a study number and identified for study purposes by that number only. The University of New Mexico has a locked office with locking cabinets in which study data will be kept. **No data will be transferred until DUA has been obtained.**

12. Economic Burden to Subjects

Research Procedures	**Number of Samples/ Procedures**	**Responsible Party**	
		Study	**3rd Party Payer or Participant**
Darbepoetin 10 micrograms/kg	1	☒	☐
Neurodevelopmental Testing	1	☒	☐
Follow up phone calls	2	☒	☐
Antibody testing	2	☒	☐
Pk Testing (non scavenged blood)	1	☒	☐
Pk Testing (scavenged blood)	2-10	☒	☐
_____	_____	☐	☐
_____	_____	☐	☐
Standard of Care Procedures	**Number of Samples/ Procedures**	**Responsible Party**	
		Study	**3rd Party Payer or Participant**
Routine neonatal care	_____	☐	☒
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐
_____	_____	☐	☐

13. Compensation

At this time there are no plans for compensation. With further funding for this project then a modification will be submitted.

14. Compensation for Research-Related Injury

If the infant is injured as a result of this study, the University of New Mexico Health Sciences Center (UNMHSC) will provide the infant with emergency treatment, at usual charge. No commitment is made by the UNMHSC to provide free medical care or money for injuries to participants in this study.

15. Consent Process

Consent

The study and its options will be explained to the parent(s) ensuring that they know this is a voluntary study and they are in no way obligated to participate. Consent will be obtained by one of the study team members. Parents will be allowed time to ask questions and will be able to read the consent document as it is discussed. Consent should be obtained by the mother, if the mother is unable to sign and she is married to the father then the father may sign consent. If consent is given, a second clinical person will verify that they have understood all that has been explained to them and they will be asked to confirm that they are allowing their child to participate in this research study. In rare instance, if a baby is transported then consent will be obtained over the phone and signed consent faxed to UNMH. Study procedures will begin only after signed consent has been received.

Subjects not fluent in English

After the English consent has been approved by the IRB we will obtain translation services in Spanish and submit at modification to the study including the Spanish consent.

Subjects who are not yet adults (infants, children, teenagers)

This study involves children from age 0 to 12 months.

Written consent will be obtained by an investigator in a room which ensures the privacy of the family, and which is free of potential coercive influences. Consent for participation must be obtained by the time the baby is 24 hours of age.

If a family has limited or no English speaking abilities, a certified interpreter will be provided. They will review the consent form with the family, and interpret the verbal explanation of the study during the discussion between the investigator and the family members. If individual sites have a large population of non-English speakers, consent forms will be translated into the appropriate languages. If an interpreter is not available in a timely manner, the family will not be approached.

The parents of the research participants will be given opportunity to review the study both verbally and in writing. The will be given opportunity to ask questions of the investigator prior to giving consent.

If there are changes in the protocol or safety information that require consent forms to be updated, they will be sent through the IRB process for approval. The study entry form has a space for documentation of a signed consent form as well as a signed HIPAA form.

16. Study Test Results/Incidental Findings

Individual Results: Families will have access to neurodevelopmental results which will be delivered at the conclusion of testing and in a formal report delivered by mail. The formal report will have a way to contact the investigators should the family have questions about the results. In addition, if the child is found to be delayed, we will facilitate the child's enrollment in therapy services.

Incidental Findings: Given that only one dose of medication is being given it is unlikely to generate any incidental findings. We will facilitate finding resources for any child that is found to have a delay

17. Data Management/Confidentiality

The data will be stored electronically, stripped of any subject identifiers. The e-data will be stored for at least 10 years after the study closes. All data is coded with a unique identifying number, different from the patient's medical record number or social security number, to maintain confidentiality. The link between identifiers and study ID will be destroyed upon closure of this study protocol with the HRRC. While identifiers are not needed in the participant data, we will have access to the patient's electronic medical record in order to retrieve study data. This means that the patients' medical record number is required to be able to locate the medical record and conduct follow-up activities. The medical record number will not be used in data analysis and all identifiers on participant data will be removed and coded with study ID. Data will be stored in RedCap which is HIPAA secure. **No data will be transferred until DUA has been obtained**

18. Data and Specimen Banking

Blood specimens will be banked for up to 3 years (approximately 6 months after the last patient is enrolled).

At the completion of the study, University of Utah will send study samples to University of New Mexico for immunogenicity and/or pK testing. Samples will be submitted per the attached Material Transfer Agreement.

Checklist Section

This section contains checklists to provide information on a variety of topics that require special determinations by the IRB. Please complete all checklists relevant to your research.

I. Waivers or Alterations of Consent, Assent, and HIPAA Authorization

A. Partial Waiver of Consent for Screening/Recruitment

Complete this checklist if you are requesting a partial waiver of consent so that you can review private information to identify potential subjects and/or determine eligibility prior to approaching potential subjects for consent or parental permission.

1. Describe the data source that you need to review (e.g., medical records):

Eligible patients will be identified by the admitting neonatologist or by the research nurses based on admission diagnosis (perinatal depression, mild NE or NE, encephalopathy, clinical assessment for the need for cooling).

2. Describe the purpose for the review (e.g., screening):

Medical charts will not be reviewed for recruitment or screening. Instead, eligible patients will be identified by the admitting neonatologist or by the research nurses based on admission diagnosis (perinatal depression, mild NE or NE, encephalopathy, clinical assessment for the need for cooling).

3. Describe who will conducting the reviews (e.g., investigators, research staff):

The admitting neonatologist or by the research nurses based on admission diagnosis (perinatal depression, mild NE or NE, encephalopathy, clinical assessment for the need for cooling)

4. Do all persons who will be conducting the reviews already have permitted access to the data source?

   ☒ Yes

   ☐ No. Explain:

5. Verify that each of the following are true or provide an alternate justification for the underlined regulatory criteria:

   a) The activity involves no more than minimal risk to the subjects because the records review itself is non-invasive and the results of the records review will not be used for any purposes other than those described above.

      ☒ True

☐ Other justification:

b) The waiver or alteration will not adversely affect the rights and welfare of the subjects because eligible subjects will be approached for consent to participate in the research and are free to decline. Further, the information accessed during the records review will not be disclosed to anyone without a legitimate purpose (e.g., verification of eligibility).

☒ True

☐ Other justification:

c) The research could not practicably be carried out without the waiver or alteration because there is no other reasonably efficient and effective way to identify who to approach for possible participation in the research.

☒ True

☐ Other justification:

d) Whenever appropriate, potentially eligible subjects will be presented with information about the research and asked to consider participation. *(Regulatory criteria: Whenever appropriate, the subjects will be provided with additional pertinent information after participation.)*

☒ True

☐ Other justification:

Partial Waiver of HIPAA Authorization for Screening/Recruitment
Complete the following additional questions/attestations if the records you will review to identify potential subjects and/or determine eligibility include Protected Health Information (PHI).

6. Will you be recording any PHI when conducting the records review to identify potential subjects and/or determine eligibility?

   ☐ Yes. Describe:

   ☒ No

7. If you answered “Yes” to question 6 above, please describe when you will destroy identifiers (must be the earliest opportunity consistent with the conduct of the research) or provide justification for why they must be retained:

8. The PHI accessed or recorded for identification/screening purposes will not be reused or disclosed to (shared with) any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of the PHI would be permitted under the Privacy Rule.

   ☒ True

   ☐ False

II. Vulnerable Populations

A. Children

Complete this checklist if the subject population will include children.

1. Select the category of research that you believe this research falls within and provide justification for any associated criteria. If there are different assessments for different groups of children or arms (e.g., placebo vs. drug), include a memo to provide an assessment for each group.

   ☐ Research not involving greater than minimal risk. *(Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.)*

   ☒ Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

   Provide justification for each of the following criteria:

   (1) The risk is justified by the anticipated benefit to the subjects:

   Darbe has the potential to improve neurodevelopmental outcomes in a group of infants with no therapeutic options at this time. Over the last decade, EPO (and its analogue darbepoetin) neuroprotective actions and underlying mechanisms in terms of signal transduction pathways have been studied in great of detail. Over 3000 infants have been treated with Erythopoesis Stimulatin Agents (ESA). The side effect profile of infants treated with ESAs is similar to those infants who received placebo.

   (2) The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches:

The pharmacokinetics of ESAs in neonatal brains are now better understood, and the safety of routine administration of ESAs to treat neonates with moderate/severe NE has been shown to be beneficial.

In the present multicenter randomized study, we anticipate that Darbe administered to neonates with mild NE who do not qualify for hypothermia will be safe and reduce long-term neurodevelopmental impairments.

☐ Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition.

Provide justification for each of the following criteria:

(1) The risk represents a minor increase over minimal risk:

(2) The intervention or procedure presents experiences to subjects that are reasonably commensurate with those inherent in their actual or expected medical, dental, psychological, social, or educational situations:

(3) The intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition which is of vital importance for the understanding or amelioration of the subjects' disorder or condition

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