High-Dose Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy: A Phase II Trial

Yvonne W. Wu, MD, MPH,[a,b] Amit M. Mathur, MD,[c] Taeun Chang, MD,[d,e] Robert C. McKinstry, MD,[f] Sarah B. Mulkey, MD, PhD,[g] Dennis E. Mayock, MD,[h] Krisa P. Van Meurs, MD,[i] Elizabeth E. Rogers, MD,[b] Fernando F. Gonzalez, MD,[b] Bryan A. Comstock, MS,[j] Sandra E. Juul, MD, PhD,[h] Michael E. Msall, MD,[k] Sonia L. Bonifacio, MD,[i] Hannah C. Glass, MDCM,[a,b,l] An N. Massaro, MD,[e] Lawrence Dong, MD,[m] Katherine W. Tan, BS,[j] Patrick J. Heagerty, PhD,[j] Roberta A. Ballard, MD[b]

abstract

OBJECTIVE: To determine if multiple doses of erythropoietin (Epo) administered with hypothermia improve neuroradiographic and short-term outcomes of newborns with hypoxic-ischemic encephalopathy.

METHODS: In a phase II double-blinded, placebo-controlled trial, we randomized newborns to receive Epo (1000 U/kg intravenously; n = 24) or placebo (n = 26) at 1, 2, 3, 5, and 7 days of age. All infants had moderate/severe encephalopathy; perinatal depression (10 minute Apgar <5, pH <7.00 or base deficit ≥15, or resuscitation at 10 minutes); and received hypothermia. Primary outcome was neurodevelopment at 12 months assessed by the Alberta Infant Motor Scale and Warner Initial Developmental Evaluation. Two independent observers rated MRI brain injury severity by using an established scoring system.

RESULTS: The mean age at first study drug was 16.5 hours (SD, 5.9). Neonatal deaths did not significantly differ between Epo and placebo groups (8% vs 19%, P = .42). Brain MRI at mean 5.1 days (SD, 2.3) showed a lower global brain injury score in Epo-treated infants (median, 2 vs 11, P = .01). Moderate/severe brain injury (4% vs 44%, P = .002), subcortical (30% vs 68%, P = .02), and cerebellar injury (0% vs 20%, P = .05) were less frequent in the Epo than placebo group. At mean age 12.7 months (SD, 0.9), motor performance in Epo-treated (n = 21) versus placebo-treated (n = 20) infants were as follows: Alberta Infant Motor Scale (53.2 vs 42.8, P = .03); Warner Initial Developmental Evaluation (28.6 vs 23.8, P = .05).

CONCLUSIONS: High doses of Epo given with hypothermia for hypoxic-ischemic encephalopathy may result in less MRI brain injury and improved 1-year motor function.

Departments of [a]Neurology, [b]Pediatrics, and [l]Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California; Departments of [c]Pediatrics and [f]Radiology, Washington University School of Medicine, St Louis, Missouri; Departments of [d]Neurology and [e]Neonatology, Children's National Health Systems, Washington, District of Columbia; [g]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas; Departments of [h]Pediatrics and [j]Biostatistics, University of Washington, Seattle, Washington; [i]Department of Pediatrics, Stanford University School of Medicine, Palo Alto, California; [k]Section of Developmental and Behavioral Pediatrics, Department of Pediatrics, University of Chicago Medicine, Comer Children's Hospital, Chicago, Illinois; and [m]Department of Pediatrics, Kaiser Permanente Santa Clara, Santa Clara, California

Dr Wu conceptualized and designed the study, coordinated and supervised data collection, performed data analyses, drafted the initial manuscript, and revised the manuscript; Drs Mathur, Chang, McKinstry, Mulkey, Mayock, Van Meurs, Rogers, Gonzalez, Comstock, Juul, Msall, Bonifacio, Glass, Massaro, Dong, Tan, Heagerty, and Ballard provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

WHAT'S KNOWN ON THIS SUBJECT: Infants with hypoxic-ischemic encephalopathy suffer a high rate (>40%) of death or moderate to severe disability, even after therapeutic hypothermia. High-dose erythropoietin reduces brain injury and improves neurologic function in animal models of neonatal hypoxic-ischemic brain injury.

WHAT THIS STUDY ADDS: Among infants undergoing hypothermia for moderate/severe hypoxic-ischemic encephalopathy, multiple high doses of erythropoietin (1000 U/kg) given intravenously over 7 days appeared safe, resulted in less MRI brain injury, and led to improved short-term motor outcomes.

To cite: Wu YW, Mathur AM, Chang T, et al. High-dose Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy: A Phase II Trial. *Pediatrics*. 2016;137(6):e20160191

Perinatal hypoxic-ischemic encephalopathy (HIE), an important cause of neonatal encephalopathy, occurs in 1 to 3 per 1000 term births[1,2] and accounts for 22% of neonatal deaths worldwide.[3] Up to 12 000 infants are affected each year in the United States. Although therapeutic hypothermia provides modest improvements in outcome,[4–8] >40% of infants who received this therapy in clinical trials either died or suffered moderate to severe disabilities, including cerebral palsy, intellectual impairment, and epilepsy.[5–9] Adjuvant neuroprotective therapies are needed to further improve outcomes after HIE.

Erythropoietin (Epo) is a cytokine that demonstrates remarkable neuroprotective and neuroregenerative effects in the brain.[10–15] In a phase I trial of combined Epo treatment with hypothermia, we found that Epo (1000 U/kg given intravenously) provided the optimal plasma Epo levels consistent with animal studies of neuroprotection.[16] Although the study was not designed to evaluate efficacy, patients who received multiple high doses of Epo exhibited a lower rate of death or moderate/severe disability at 22 months (4.5%)[17] than had been expected based on studies of infants with similar entry criteria who received hypothermia alone (44%–51%).[4–6,8]

Two small trials in China and Egypt found that Epo therapy improved short-term neurologic outcomes after HIE.[18,19] However, these trials used alternative dosing regimens and did not include the use of hypothermia. In countries where therapeutic hypothermia has become a standard of care,[20–22] novel neuroprotective agents should be evaluated together with hypothermia.[23] Epo is commercially available, easy to administer, and has a good safety profile in newborns.[24] To further evaluate Epo as a potential neuroprotective agent for HIE, we performed a phase II trial to compare early developmental outcomes in patients treated with Epo plus hypothermia with those who received hypothermia alone.

TABLE 1 Severity of Encephalopathy, Based on Modified Sarnat Scoring System

	Moderate	Severe
Consciousness	Decreased	Absent
Spontaneous activity	Decreased	No activity
Tone	Hypotonia	Flaccid
Suck	Weak	Absent
Moro	Incomplete	Absent
Respiration	Periodic breathing	Apnea

Encephalopathy = at least 3 of 6 criteria present. Severe = more symptoms in the severe than moderate column. Moderate = more symptoms in the moderate column. If encephalopathy signs were equally distributed between moderate and severe categories, severity of encephalopathy was based on level of consciousness.[4]

METHODS

In a multicenter, double-blinded, placebo-controlled trial (Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes, or "NEATO"), we enrolled 50 newborns with moderate/severe HIE at 7 centers: Children's National Health System (n = 9); University of California, San Francisco (n = 9); Seattle Children's Hospital (n = 8); Arkansas Children's Hospital (n = 8); Washington University, St Louis (n = 8); Stanford University (n = 6); and Kaiser Permanente Santa Clara (n = 2). The study received institutional review board approval at all hospitals, was overseen by an independent data and safety monitoring board (DSMB), and was registered with the US Food and Drug Administration (Investigational New Drug 102 138).

Patient Selection

Participants met 4 inclusion criteria: (1) ≥36 weeks gestational age; (2) whole-body hypothermia (n = 42) or selective head cooling (n = 8) initiated by 6 hours of age; (3) perinatal depression with at least 1 of the following: 10 minute Apgar score <5; need for chest compressions or endotracheal/mask ventilation at 10 minutes; pH <7.00 or base deficit ≥15 in cord or arterial blood within 60 minutes of birth; and (4) moderate/severe encephalopathy evident by at least 3 of 6 modified Sarnat criteria present between 1 to 6 hours of age, as defined in Table 1.[4,6,25] We evaluated severity of encephalopathy at baseline and at 5 and 7 days of age.

We excluded patients with any of the following: age at time of consent >23.5 hours; congenital anomaly; suspected genetic syndrome; birth weight <1800 g; head circumference <2 SDs below the mean; no indwelling line; withdrawal of care being considered because of moribund condition; or unlikely to obtain follow-up at 12 months of age.

Intervention

After consent was obtained, participants were randomized to receive either Epo (1000 U/kg intravenously) or an equal volume of normal saline on days 1, 2, 3, 5, and 7. The first study drug dose was given as soon as possible, up to 24 hours of age. Pharmacists assigned participants to treatment groups by using a randomization table created by a biostatistician. Randomization was stratified by site and severity of encephalopathy. All participants, study personnel other than pharmacists and biostatisticians, and all outcome assessors were blinded to treatment assignment.

Neurodevelopmental Outcome

Primary outcome was determined a priori to be 12-month neurodevelopment assessed by

(1) Alberta Infant Motor Scale (AIMS),[26–30] a standardized and validated exam that objectively rates motor function, and (2) the Warner Initial Developmental Evaluation (WIDEA), a 43-item parental questionnaire that assesses 4 domains of infant development: self-care, mobility, communication, and social cognition.[31,32] The WIDEA was also administered at 6 months of age. Moderate to severe neurodevelopmental impairment at 12 months was defined as an AIMS score less than the fifth percentile for age, or a WIDEA score >2 SDs below the mean based on normative data from typically developing infants at 12.9 months of age (ie, WIDEA <76.4).[33] All AIMS and WIDEA evaluators underwent centralized training to maximize consistency across sites.

Safety

Serious adverse events required expedited review by an independent DSMB: (1) in-hospital death; (2) severe cardiopulmonary collapse requiring cardiopulmonary resuscitation within 2 hours of study drug; (3) thrombosis of a major vessel; or (4) unexpected events thought to be related to the study drug. We also recorded adverse events that are frequent comorbidities of HIE: disseminated intravascular coagulation with clinical bleeding requiring transfusion; hypotension requiring inotrope or vasopressor support; hypertension requiring antihypertensive medication; liver injury (alanine aminotransferase >100 IU/L); persistent pulmonary hypertension requiring nitric oxide and fraction of inspired oxygen >0.50; platelet count <100 000 per μL; creatinine >1.5 mg/dL; sepsis (positive blood culture and at least 7 days of antibiotic treatment); and polycythemia requiring intervention.

Neuroimaging

Neonatal brain MRI was performed as part of routine clinical care at 4 to 7 days of age. This time window minimizes the likelihood of diffusion-weighted imaging pseudonormalization, and follows the American Academy of Neurology practice parameters.[34–36] Two central reviewers (R.M. and A.M.), who were blinded to treatment allocation, independently scored brain MRI findings as previously described,[34,] resolving discrepancies through discussion. Global brain injury score (range, 0–138) was determined by measuring injury severity (0 = normal, 3 = severe) in 8 brain regions in each hemisphere (caudate, putamen/globus pallidus, thalamus, posterior limb of internal capsule, white matter, cortex, brainstem, and cerebellum) and adding these component scores together.[34] Severity of brain injury was categorized as "none" = 0; "mild" = 1–11; "moderate" = 12–32; or "severe" = 33–138.

Statistical Analyses

For primary analysis of treatment effect, we used an intention-to-treat strategy. We calculated the effect of Epo on developmental outcome measures, 95% confidence intervals, and corresponding *P* values (significance, $P < .05$) by using a linear regression with robust SEs, with and without adjusting for age at testing and for severity of encephalopathy at baseline. We compared categorical variables between randomized treatment groups by using a χ^2 test, or Fisher's exact test when the count was ≤5. To compare baseline continuous variables between treatment groups, we performed a two-sided *t* test with unequal variances, and a Wilcoxon rank sum test for the global brain injury score because these data were right-skewed. We used a Poisson regression model with robust SEs adjusting for severity of encephalopathy to compare adverse event and serious adverse event counts between treatment groups. The sample size was determined to provide sufficient evidence regarding the equivalence or noninferiority of Epo relative to the placebo for key safety measures, with 50 subjects yielding 80% power to evaluate equivalence when using tolerance limits of 0.5 SDs for quantitative measures of organ function. The sample size of 50 was also considered adequate to evaluate feasibility for a future phase III trial. Neuroimaging biomarkers were explored as secondary outcomes, and thus no corrections were made for multiple comparisons. All statistical analyses were performed by an independent biostatistician by using R statistical software (version 3.2, The R Foundation, Vienna, Austria).[37] All reported *P* values are 2-sided.

RESULTS

Of 154 newborns with HIE who were evaluated from January to November 2012, 81 (53%) were eligible and 68 were approached for consent (Fig 1). Fifty (74% consent rate) were randomly allocated to receive Epo with hypothermia ($n = 24$) or hypothermia alone ($n = 26$). The first study drug dose was administered at a mean age of 16.5 hours (SD, 5.9). Forty (80%) infants received all 5 doses; the remaining 10 did not because they died before completing treatment ($n = 5$), lost intravenous access ($n = 3$), or were discharged to home before 7 days ($n = 2$). Of the 24 infants in the Epo group, 22 (92%) received all 5 doses, 1 (4%) received 4 doses, and 1 (4%) only received 1 dose due to redirection of care.

The 2 treatment groups were similar with respect to baseline characteristics (Table 2), except for a higher frequency of large for gestational age infants in the Epo group (25% vs 4%, $P = .04$). All infants were singleton gestation.

Severity of encephalopathy was not significantly different between the groups.

Neonatal Outcomes

Death during neonatal hospitalization occurred in 7 (14%) patients, and did not differ significantly between treatment groups (8% vs 19%, P = .42). Death was more common after severe encephalopathy compared with moderate encephalopathy (44% vs 7%, P = .02). All deaths were attributed to redirection of care due to critical medical condition (n = 3), poor neurologic prognosis (n = 1), or both (n = 3). Moderate/severe encephalopathy based on Sarnat exam had resolved by 5 days in a larger proportion of infants treated with Epo than those treated with the placebo (61% vs 32%, P = .045); this difference was no longer significant at 7 days (67% vs 48%, P = .13).

Neurodevelopmental Outcome

Forty-one (82%) subjects survived and underwent a 12-month evaluation (Fig 1). Of 43 survivors, 41 (95%) were successfully evaluated at a mean age of 12.7 months (SD, 0.9). Age at follow-up did not differ between treatment groups (Table 3). No patients died between hospital discharge and final follow-up. At 12 months, the AIMS evaluation revealed a significantly higher score in the Epo group compared with the placebo group (53.5 vs 42.8, P = .02), whereas the WIDEA score showed a trend toward improvement in the Epo group (122 vs 110, P = .10). The 6-month WIDEA score was higher in the Epo group (75.3 vs 68.8, P = .04). After adjusting for age at testing and severity of encephalopathy, these differences did not appreciably change (Table 3).

Additional exploratory analyses were conducted, but not corrected for multiple comparisons because they were not considered confirmatory endpoints in this phase II trial. The WIDEA mobility subscore was higher

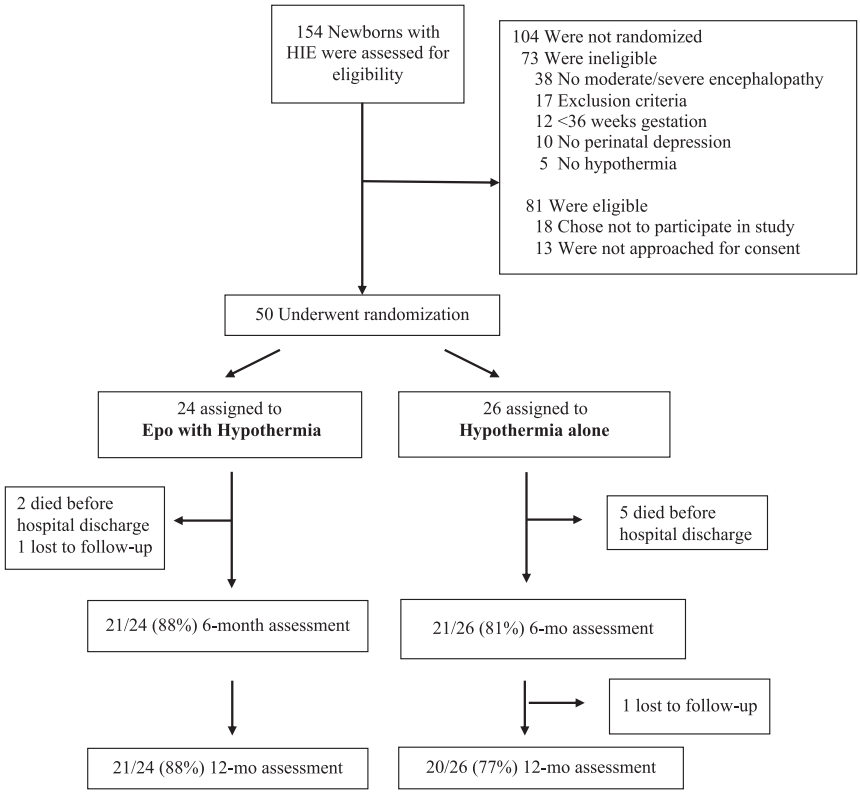

FIGURE 1
Evaluated newborns with HIE.

in the Epo group at 12 months (28.6 vs 23.8, adjusted P = .048). When comparing the composite outcome of death or moderate to severe neurodevelopmental impairment at 12 months in Epo and placebo groups (16.7% vs 38.5%, P = .12), there was a nonsignificant trend toward benefit with treatment.

Safety

No adverse events were attributed to Epo. Expected adverse events were common and evenly distributed (Table 4), except for a higher rate of sepsis in the placebo group. No patients in either group developed polycythemia. The mean, final hematocrit recorded at a mean age of 5.7 days did not differ between the Epo and placebo groups (37.1 vs 38.4, P = .53).

Serious adverse events occurred in 9 patients, and were seen in both treatment groups (Table 4). In addition to the 7 hospital deaths, 1 patient in each group developed a deep vein thrombosis, and 1 infant required cardiac compressions and intubation within 2 hours of receiving Epo; however, the cardiopulmonary compromise was in the setting of severe multiorgan injury and was considered by the DSMB to be unlikely related to the study drug.

Neuroimaging

Brain MRI was performed in 48 (96%) infants at a mean age of 5.2 days (SD, 2.2). Two patients died before neuroimaging was obtained. Of the 23 patients in the Epo group who had a brain MRI, all had received at least 3 doses of the study drug before undergoing neuroimaging. Global brain injury score on MRI ranged from 0 to 70 (interquartile range [IQR], 1–11). Infants who received Epo had significantly lower global brain injury scores (median, 2; IQR, 0–8.5) than those in the placebo

TABLE 2 Baseline Characteristics of Study Participants by Treatment Group

	Epo (n = 24)		Placebo (n = 26)		P[a]
Baseline Characteristics	n	n (%)	n	n (%)	
Maternal race	24		26		.49
White		14 (58)		18 (69)	
Black		7 (29)		4 (15)	
Asian		2 (8)		1 (4)	
Other		1 (4)		3 (12)	
Maternal Hispanic ethnicity	24	5 (20.8)	26	6 (23.1)	.85
Maternal age, mean (SD)	24	29.4 (7.2)	26	29.7 (6.6)	.87
Maternal education	21		24		.82
High school or less		10 (47)		10 (42)	
Some college or university		5 (24)		7 (29)	
College graduate, or post-graduate		6 (29)		7 (29)	
Female infant	24	14 (58)	26	10 (39)	.26
Birth weight, g, mean (SD)	24	3556 (618)	26	3243 (512)	.06
Gestational age, wks, mean (SD)	23	38.7 (1.9)	26	38.7 (1.6)	.99
Large for gestational age	24	6 (25)	26	1 (4)	.04
Severe Sarnat encephalopathy[b]	24	5 (21)	26	4 (15)	.72
5 min Apgar	23		25		.74
0–3		11 (48)		14 (56)	
4–6		10 (43)		10 (40)	
7–10		2 (9)		1 (4)	
10 min Apgar	21		25		.39
0–3		5 (24)		7 (28)	
4–6		13 (62)		11 (44)	
7–10		3 (14)		7 (28)	
Resuscitation >10 min[c]	24	21 (88)	26	21 (81)	.70
Chest compressions	24	8 (33)	26	11 (42)	.72
Lowest pH[d], mean (SD)	20	6.9 (0.2)	24	7.0 (0.2)	.47
aEEG severe abnormal background[e]	22	7 (32)	24	7 (29)	.85
Delivery mode	24		26		.50
Spontaneous vaginal		5 (21)		9 (34)	
Vacuum or forceps		1 (4)		2 (8)	
Elective cesarean		1 (4)		0 (0)	
Emergency cesarean		17 (71)		15 (58)	
Maternal chorioamnionitis	24	4 (17)	26	3 (12)	.70
Sentinel event[f]	24	7 (29)	26	7 (27)	.86
Age at randomization, h, mean (SD)	24	13.3 (6.1)	24	14.6 (6.1)	.48
Age at first study drug, h, mean (SD)	24	15.6 (5.7)	26	17.2 (6.1)	.35

[a] P values for categorical variables are based on 2-sided χ^2 or Fisher's exact test as appropriate. For continuous variables, P values are based on 2-sided t test with unequal variances.
[b] Severe encephalopathy as defined in Table 1.
[c] Required ongoing resuscitation with chest compressions and/or mechanical ventilation at 10 min of age.
[d] Lowest pH among cord arterial, cord venous, and arterial blood gas samples taken before 60 min of age.
[e] Severe amplitude-integrated electroencephalography (aEEG) background at baseline, defined as burst suppression, continuous low voltage, or inactive flat tracing.
[f] Sentinel event = placental abruption, shoulder dystocia, uterine rupture, or prolapsed cord.

group (median, 11; IQR, 4–18, P = .01; Table 5). Fewer Epo-treated infants had moderate to severe brain injury on MRI (4% vs 44%, P = .002). There was a trend toward more normal brain MRIs among patients who received Epo (35% vs 12%, P = .09). Injury to subcortical regions of the brain (ie, basal ganglia, thalamus, or posterior limb of the internal capsule) was significantly less common in the Epo than in the placebo group (30% vs 68%, P = .02). Cerebellar injury was also less common in the Epo group (0% vs 20%, P = .051).

Sensitivity Analyses

Infant birth weight differed between the treatment groups (P = .06, Table 2); when adjusted for birth weight, the effect of treatment on MRI global injury score and on neurodevelopmental outcomes did not appreciably change. After randomization, 2 patients in the placebo group were diagnosed with conditions that would have excluded them from the study (ie, myotonic dystrophy and brainstem malformation). When we excluded these patients from the analyses, the mean increases in the 12-month AIMS (6.8, P = .09) and WIDEA (5.3, P = .42) scores associated with Epo treatment were not statistically significant; however, the significant effect of treatment on MRI global injury score remained unchanged. None of the WIDEA or AIMS scores showed treatment moderation by gender.

TABLE 3 Neurodevelopmental Outcomes and Growth at 6 and 12 mo of Age

Outcome	Epo	Placebo	Adjusted Treatment Effect (95% Confidence Interval)[a]	*P*[a]
6 mo	*n* = 21	*n* = 21		
WIDEA				
Age at testing, mo	6.3 (0.6)[b]	6.1 (0.4)	NA	.34[c]
Total score	75.3 (9.1)	68.8 (10.7)	6.7 (0.69 to 12.8)	.04
Self-care	28.1 (4.2)	26.1 (4.7)	1.8 (−0.97 to 4.6)	.20
Mobility	14.1 (2.7)	12.4 (2.7)	1.5 (0.07 to 3.00)	.06
Communication	16.4 (3.2)	15.3 (2.8)	1.2 (−0.63 to 3.1)	.20
Social	16.7 (4.5)	14.9 (3.2)	2.1 (−0.12 to 4.3)	.09
12 mo	*n* = 21	*n* = 20		
WIDEA				
Age at testing, mo	12.7 (0.9)	12.6 (0.9)	NA	.71[c]
Total score	122 (14)	110 (31)	10.8 (−2.8 to 24.5)	.15
Self-care	36.7 (5.1)	33.8 (7.7)	2.8 (−1.1 to 6.8)	.18
Mobility	28.6 (3.8)	23.8 (8.9)	4.4 (0.46 to 8.37)	.048
Communication	28.2 (5.1)	25.5 (8.8)	2.2 (−1.9 to 6.3)	.33
Social	28.8 (6.4)	26.9 (8.9)	1.4 (−3.1 to 5.9)	.57
AIMS	53.5 (5.2)	42.8 (19.3)	10.2 (1.9 to 18.5)	.03
Moderate to severe NDI[d], *n* (%)	2 (8)	5 (19)	NA	.42
Weight, kg	9.9 (1.4)	9.7 (1.2)	0.06 (−0.74 to 0.86)	.88
Height, cm	74.3 (3.2)	71.7 (6.2)	2.2 (−0.81 to 5.3)	.17
Head circumference, cm	45.7 (1.6)	45.5 (2.1)	0.2 (−1.0 to 1.4)	.75

[a] Treatment effect comparing Epo to Placebo is based on linear regression adjusted for age at testing and severity of encephalopathy. 95% confidence intervals and corresponding 2-sided *P* values are based on robust (sandwich) SEs.
[b] Measurements are provided as mean (SD).
[c] *P* values for differences in age at testing are based on a *t* test of difference in means with unequal variances.
[d] Moderate to severe neurodevelopmental impairment (NDI) among survivors at age 12 mo, defined as AIMS less than fifth percentile for age, or WIDEA <2 SDs below the mean. *P* value is based on Fisher's exact test.

TABLE 4 Adverse Events and Significant Adverse Events by Treatment Group

	Epo (*n* = 24)	Placebo (*n* = 26)	*P*[a]
Adverse events, *n* (%)			
Liver dysfunction (alanine aminotransferase >100 IU/L)	10 (42%)	6 (23%)	.16
Hypotension (requiring inotrope or vasopressor)	9 (38%)	12 (46%)	.74
Thrombocytopenia (platelet <100 000 per μL)	6 (25%)	10 (39%)	.31
Persistent pulmonary hypertension	5 (21%)	4 (15%)	.89
Disseminated intravascular coagulation	3 (13%)	5 (19%)	.70
Sepsis (positive blood culture and antibiotics ≥7 d)	0 (0%)	5 (19%)	.051
Renal dysfunction (creatinine >1.5)	4 (17%)	5 (19%)	.81
Hypertension (requiring antihypertensive)	1 (4%)	0 (0%)	.48
Polycythemia (requiring intervention)	0 (0%)	0 (0%)	.99
Total adverse events, *n* (*n* per patient)	38 (1.6)	47 (1.8)	.51[b]
Patients with ≥1 adverse event, *n* (%)	17 (71%)	16 (62%)	.49
Serious adverse events, *n* (%)			
Death during birth hospitalization	2 (8%)	5 (19%)	.42
Cardiopulmonary collapse within 2 h of drug	1 (4%)	0 (0%)	.48
Thrombosis of major vessel	1 (4%)	1 (7%)	.99
Unexpected event related to study drug	0 (0%)	0 (0%)	.99
Any of the above	3 (13%)	6 (23%)	.47
Total serious adverse events, *n* (*n* per patient)	4 (0.17)	6 (0.23)	.43[b]

[a] *P* values are based on 2-sided χ^2 or Fisher's exact test as appropriate.
[b] *P* values are based on robust SEs using a Poisson regression of counts on treatment, adjusting for encephalopathy severity.

DISCUSSION

In this phase II multicenter, double-blinded controlled trial, we found that infants with moderate/severe HIE who received Epo as an adjunctive therapy to hypothermia demonstrated reduced severity of brain injury on neonatal MRI, and improved short-term motor outcomes. Our results suggest that treatment with multiple high doses of Epo, combined with hypothermia, is feasible, safe, and may provide further neuroprotection for moderate/severe HIE. This study also demonstrates the feasibility of performing a multicenter neonatal neuroprotection trial with a high rate

TABLE 5 Neonatal Brain MRI Findings by Treatment Group

Outcome	Epo (n = 23)	Placebo (n = 25)	P[a]
Age at MRI, days, mean (SD)	5.6 (2.8)	4.9 (1.4)	.28
Number of doses before MRI, mean (SD)	3.7 (0.8)	3.4 (0.8)	.25
Global brain injury score, *n* (%)			.01
None, 0	8 (35%)	3 (12%)	
Mild, 1–11	14 (61%)	11 (44%)	
Moderate, 12–31	0 (0%)	6 (24%)	
Severe, ≥32	1 (4%)	5 (20%)	
Median [IQR]	2 [0–9]	11 [4–18]	.01[b]
Mean (SD)	5.26 (9.9)	16.36 (18.3)	
Presence of brain injury, by region[c]			
Subcortical[d]	7 (30%)	17 (68%)	.02
Cortical	4 (17%)	9 (36%)	.26
White matter	12 (52%)	15 (60%)	.80
Brainstem	1 (4%)	4 (16%)	.35
Cerebellar	0 (0%)	5 (20%)	.051
≥2 regions injured	7 (30%)	14 (56%)	.14

[a] P values for categorical variables are based on 2-sided χ^2 or Fisher's exact test as appropriate.
[b] P value is based on a Wilcoxon rank sum test.
[c] For each region, brain injury was considered to be present if the MRI injury subscore for that region was >0.
[d] Subcortical injury includes injury to the basal ganglia, thalamus, or posterior limb of the internal capsule.

of follow-up (ie, 95% of survivors) at 1 year.

Epo receptors are expressed in the brain on numerous cell types including neuronal progenitors,[38] mature neurons,[39] astrocytes,[40] oligodendrocytes,[40] and microglia.[41] Epo exhibits antiapoptotic and antiinflammatory effects acutely after neonatal brain injury[42–46] and promotes neurogenesis, plasticity, and tissue remodeling after hypoxia-ischemia.[15,47–50] In animal models of neonatal stroke, Epo increases proliferation, migration, and differentiation of neuronal precursors, resulting in increased neurogenesis in the injured basal ganglia and cortex.[47,51,52]

Although therapeutic hypothermia has improved the outlook of infants with HIE,[53,54] there remains a pressing need for neuroprotective therapies that will further reduce the high rate of neurologic disabilities.[55–57] We reported the safety and pharmacokinetics of high-dose Epo when given together with hypothermia.[16,17] Darbepoetin, a long-acting formulation of Epo, has also been shown to be safe in newborns undergoing hypothermia for HIE.[58] Epo monotherapy, without hypothermia, may be useful for neonatal conditions other than HIE, such as perinatal stroke,[59] congenital heart disease,[60] and brain injury of prematurity.[61–64]

This is the first clinical study of HIE that assesses biomarkers of efficacy to evaluate whether Epo provides additional neuroprotection to hypothermia. We found that Epo treatment was associated with significantly reduced severity of brain injury on MRI, specifically in the subcortical region (ie, the area that contains the basal ganglia, thalamus, and internal capsule). In term infants, the subcortical region of the brain exhibits selective neuronal vulnerability to hypoxia-ischemia.[65] Thus, our findings suggest that Epo specifically reduces injury to the areas of the brain that are most susceptible to HIE.

Subcortical brain injury is associated with a particularly high risk for later motor disability.[66,67] Because the Epo group had a lower incidence of subcortical injury on brain MRI, it is not surprising that Epo treatment also resulted in improved motor outcomes. Although our study was not designed to evaluate long-term outcomes, such as cerebral palsy, Epo resulted in improved short-term biomarkers of motor outcome, as measured by observed motor evaluation (AIMS) and parental questionnaire (WIDEA) at 12 months. What constitutes a clinically significant difference in these scores at 12 months is not well established. However, the average difference in AIMS motor scores between the Epo and placebo groups (ie, 10.2 points) is equal to more than one-half of 1 SD of the scores in the placebo group (ie, 19.3 points), suggesting a relatively large effect size.

Epo may improve neurologic outcomes by acutely reducing the degree of brain injury after hypoxia-ischemia, by improving repair through its long-term effects on neuronal regeneration, or both.[43,68] In our study, early brain MRI performed at a mean age of 5 days detected a significantly reduced amount of injury among infants who had received ≥3 doses of Epo. Furthermore, at 5 days of age, moderate/severe encephalopathy had resolved in a greater proportion of infants receiving ≥3 doses of Epo. Thus, our findings suggest that Epo exerts an acute neuroprotective effect when given in high doses during the first 3 days after birth. Animal studies have found that Epo administered in a delayed fashion

enhances brain repair.[15,69] Whether later doses of Epo given to infants with HIE on days 5 and 7 exert additional neuroprotection remains to be determined.

The risk of serious and expected adverse events did not differ significantly between the 2 treatment groups. No patients developed polycythemia, which is consistent with the frequent phlebotomy required to treat such critically ill newborns. Although the Epo group had half as many deaths as the placebo group, this finding did not reach statistical significance given the small number of participants. Reassuringly, the rate of moderate to severe neurodevelopmental impairment at 12 months was no higher in the Epo than in the placebo group, suggesting that any deaths that might have been prevented by Epo were unlikely to have led to a greater severity of neurodevelopmental abnormalities in surviving infants.

The relatively small size of this phase II trial is an important limitation. After post-hoc exclusion of 2 patients who later met exclusion criteria, the apparent benefit of Epo on 12-month outcomes was no longer statistically significant. Without a standardized approach to EEG data collection, we were limited in our ability to accurately diagnose clinical and electrographic seizures across all sites. Similarly, we were unable to compare MR spectroscopy and diffusion tensor imaging measures across centers due to lack of uniform data collection procedures. Our findings require confirmation in a larger study with an adequate sample size to mitigate bias resulting from unavoidable chance confounding, with a longer period of follow-up to allow for the evaluation of long-term impacts, and with standardized neuroimaging and electrophysiological data collection across sites.

CONCLUSIONS

Among infants undergoing therapeutic hypothermia for HIE, multiple doses of Epo (1000 U/kg) given intravenously over 7 days may result in less MRI brain injury and may lead to improved short-term motor outcomes. Plans are underway to perform a large phase III trial to determine whether Epo treatment in conjunction with hypothermia improves the long-term neurologic outcome of infants with HIE.

ACKNOWLEDGMENTS

The following collaborators, in addition to those listed as authors, also participated in this study: Arkansas Children's Hospital: Andrea Ross, RN, Holly Pettit, RN, Nupur Dutta Chowdhury, MA, CCRP, and Billy Furgerson, PharmD; Children's National Health System: Avital Cnaan, PhD, Adrienne Arrieta, MS, James He, MS, and Kari Harris, MPH; Kaiser Permanente Santa Clara: Andrea Wickremasinghe; Stanford University: Anne DeBattista, PhD, C-PNP, C-PMHS, M. Bethany Ball, BS, CCRC, and Melinda Proud, RCP; University of California, San Francisco: Donna Ferriero, MD, Colin Partridge, MD, Rebecca Webb, BS, and the University of California, San Francisco investigational drug service; University of Washington: Amy E. Silvia, ScM, Elizabeth L. Howland, Kathleen Washington, PhD, PT, and Niranjana Natarajan, MD; Washington University, St Louis: Anthony Barton.

Study data were collected and managed by using REDCap electronic data capture tools hosted at University of California, San Francisco.

We thank the DSMB members Robin Ohls, MD (Chair), John Barks, MD, and Janet Soul, MD, and the Independent Medical Monitor, Maureen Gilmore, MD, for their work. We thank Jessica Kan Vedder, BA MPH, for her organizational support of the study, for creating the study database, and for her detailed work in monitoring enrollment and data collection procedures. We also thank all the patients, families, and bedside nurses who participated in this study.

ABBREVIATIONS

AIMS: Alberta Infant Motor Scale
DSMB: data and safety monitoring board
Epo: erythropoietin
HIE: hypoxic-ischemic encephalopathy
IQR: interquartile range
WIDEA: Warner Initial Developmental Evaluation

This trial has been registered at www.clinicaltrials.gov (identifier NCT 01913340).

DOI: 10.1542/peds.2016-0191

Accepted for publication Mar 1, 2016

Address correspondence to Yvonne Wu, MD, MPH, Department of Neurology, University of California, San Francisco, 675 Nelson Rising Ln, Suite 411, Box 0663, San Francisco, CA 94143. E-mail: wuy@ucsf.edu

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: This study was funded by the Thrasher Research Fund.

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

1. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev*. 2010;86(6):329–338
2. Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. A systematic review of the role of intrapartum hypoxia-ischemia in the causation of neonatal encephalopathy. *Am J Obstet Gynecol*. 2008;199(6):587–595
3. Black RE, Cousens S, Johnson HL, et al; Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375(9730):1969–1987
4. Shankaran S, Laptook AR, Ehrenkranz RA, et al; National Institute of Child Health and Human Development Neonatal Research Network. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. 2005;353(15):1574–1584
5. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet*. 2005;365(9460):663–670
6. Jacobs SE, Morley CJ, Inder TE, et al; Infant Cooling Evaluation Collaboration. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med*. 2011;165(8):692–700
7. Simbruner G, Mittal RA, Rohlmann F, Muche R; neo.nEURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics*. 2010;126(4). Available at: www.pediatrics.org/cgi/content/full/126/4/e771
8. Azzopardi DV, Strohm B, Edwards AD, et al; TOBY Study Group. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med*. 2009;361(14):1349–1358
9. Shankaran S, Pappas A, McDonald SA, et al; Eunice Kennedy Shriver NICHD Neonatal Research Network. Childhood outcomes after hypothermia for neonatal encephalopathy. *N Engl J Med*. 2012;366(22):2085–2092
10. Juul S. Erythropoietin in the central nervous system, and its use to prevent hypoxic-ischemic brain damage. *Acta Paediatr Suppl*. 2002;91(438):36–42
11. Juul SE, McPherson RJ, Bammler TK, Wilkerson J, Beyer RP, Farin FM. Recombinant erythropoietin is neuroprotective in a novel mouse oxidative injury model. *Dev Neurosci*. 2008;30(4):231–242
12. Juul S. Recombinant erythropoietin as a neuroprotective treatment: in vitro and in vivo models. *Clin Perinatol*. 2004;31(1):129–142
13. Demers EJ, McPherson RJ, Juul SE. Erythropoietin protects dopaminergic neurons and improves neurobehavioral outcomes in juvenile rats after neonatal hypoxia-ischemia. *Pediatr Res*. 2005;58(2):297–301
14. Dame C, Juul SE, Christensen RD. The biology of erythropoietin in the central nervous system and its neurotrophic and neuroprotective potential. *Biol Neonate*. 2001;79(3-4):228–235
15. Reitmeir R, Kilic E, Kilic U, et al. Post-acute delivery of erythropoietin induces stroke recovery by promoting perilesional tissue remodelling and contralesional pyramidal tract plasticity. *Brain*. 2011;134(pt 1):84–99
16. Wu YW, Bauer LA, Ballard RA, et al. Erythropoietin for neuroprotection in neonatal encephalopathy: safety and pharmacokinetics. *Pediatrics*. 2012;130(4):683–691
17. Rogers EE, Bonifacio SL, Glass HC, et al. Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatr Neurol*. 2014;51(5):657–662
18. Zhu C, Kang W, Xu F, et al. Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. *Pediatrics*. 2009;124(2). Available at: www.pediatrics.org/cgi/content/full/124/2/e218
19. Elmahdy H, El-Mashad AR, El-Bahrawy H, El-Gohary T, El-Barbary A, Aly H. Human recombinant erythropoietin in asphyxia neonatorum: pilot trial. *Pediatrics*. 2010;125(5). Available at: www.pediatrics.org/cgi/content/full/125/5/e1135
20. Perlman JM, Wyllie J, Kattwinkel J, et al; Neonatal Resuscitation Chapter Collaborators. Neonatal resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations. *Pediatrics*. 2010;126(5). Available at: www.pediatrics.org/cgi/content/full/126/5/e1319
21. Kattwinkel J, Perlman JM, Aziz K, et al. Part 15: neonatal resuscitation: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation*. 2010;122(18 suppl 3):S909–S919
22. Azzopardi D, Strohm B, Linsell L, et al; UK TOBY Cooling Register. Implementation and conduct of therapeutic hypothermia for perinatal asphyxial encephalopathy in the UK--analysis of national data. *PLoS One*. 2012;7(6):e38504
23. Davidson JO, Wassink G, van den Heuij LG, Bennet L, Gunn AJ. Therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy–Where to from here? *Front Neurol*. September 2015;6:198
24. Ohls RK, Christensen RD, Widness JA, Juul SE. Erythropoiesis stimulating agents demonstrate safety and show promise as neuroprotective agents in neonates. *J Pediatr*. 2015;167(1):10–12
25. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol*. 1976;33(10):696–705
26. Yıldırım ZH, Aydınlı N, Ekici B, Tatlı B, Calişkan M. Can Alberta Infant Motor

Scale and milani comparetti motor development screening test be rapid alternatives to bayley scales of infant development-II at high-risk infants. *Ann Indian Acad Neurol*. 2012;15(3):196–199

27. Darrah J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. *Dev Med Child Neurol*. 1998;40(7):485–491
28. Piper MC, Pinnell LE, Darrah J, Maguire T, Byrne PJ. Construction and validation of the Alberta Infant Motor Scale (AIMS). *Can J Public Health*. 1992;83(suppl 2):S46–S50
29. Darrah J, Redfern L, Maguire TO, Beaulne AP, Watt J. Intra-individual stability of rate of gross motor development in full-term infants. *Early Hum Dev*. 1998;52(2):169–179
30. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol*. 2008;50(4):254–266
31. Msall ME. Measuring functional skills in preschool children at risk for neurodevelopmental disabilities. *Ment Retard Dev Disabil Res Rev*. 2005;11(3):263–273
32. Msall M, Tremont MR, Ottenbacher KJ. Functional assessment of preschool children: optimizing developmental and family supports in early intervention. *Infants Young Child*. 2001;14(1):46–66
33. Park JJ. *Development of a Functional Assessment Tool in Children Birth to 36 Months: Validation Study of the Warner Initial Developmental Evaluation of Adaptive and Functional Skills (Warner IDEA-FS) in Typical and Atypical Children*. Chicago, IL: Liberal Arts Program, Graham School of Continuing Liberal and Professional Studies, University of Chicago; 2010
34. Bednarek N, Mathur A, Inder T, Wilkinson J, Neil J, Shimony J. Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy. *Neurology*. 2012;78(18):1420–1427
35. Barkovich AJ, Miller SP, Bartha A, et al. MR imaging, MR spectroscopy, and diffusion tensor imaging of sequential studies in neonates with encephalopathy. *AJNR Am J Neuroradiol*. 2006;27(3):533–547
36. Ment LR, Bada HS, Barnes P, et al. Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*. 2002;58(12):1726–1738
37. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2014
38. Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke*. 2004;35(7):1732–1737
39. Wallach I, Zhang J, Hartmann A, et al. Erythropoietin-receptor gene regulation in neuronal cells. *Pediatr Res*. 2009;65(6):619–624
40. Sugawa M, Sakurai Y, Ishikawa-Ieda Y, Suzuki H, Asou H. Effects of erythropoietin on glial cell development; oligodendrocyte maturation and astrocyte proliferation. *Neurosci Res*. 2002;44(4):391–403
41. Chong ZZ, Kang JQ, Maiese K. Erythropoietin fosters both intrinsic and extrinsic neuronal protection through modulation of microglia, Akt1, Bad, and caspase-mediated pathways. *Br J Pharmacol*. 2003;138(6):1107–1118
42. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. *Nature*. 2001;412(6847):641–647
43. Xiong T, Qu Y, Mu D, Ferriero D. Erythropoietin for neonatal brain injury: opportunity and challenge. *Int J Dev Neurosci*. 2011;29(6):583–591
44. Kellert BA, McPherson RJ, Juul SE. A comparison of high-dose recombinant erythropoietin treatment regimens in brain-injured neonatal rats. *Pediatr Res*. 2007;61(4):451–455
45. Sun Y, Calvert JW, Zhang JH. Neonatal hypoxia/ischemia is associated with decreased inflammatory mediators after erythropoietin administration. *Stroke*. 2005;36(8):1672–1678
46. Juul SE, Beyer RP, Bammler TK, McPherson RJ, Wilkerson J, Farin FM. Microarray analysis of high-dose recombinant erythropoietin treatment of unilateral brain injury in neonatal mouse hippocampus. *Pediatr Res*. 2009;65(5):485–492
47. Iwai M, Cao G, Yin W, Stetler RA, Liu J, Chen J. Erythropoietin promotes neuronal replacement through revascularization and neurogenesis after neonatal hypoxia/ischemia in rats. *Stroke*. 2007;38(10):2795–2803
48. Ransome MI, Turnley AM. Systemically delivered Erythropoietin transiently enhances adult hippocampal neurogenesis. *J Neurochem*. 2007;102(6):1953–1965
49. Wang L, Chopp M, Gregg SR, et al. Neural progenitor cells treated with EPO induce angiogenesis through the production of VEGF. *J Cereb Blood Flow Metab*. 2008;28(7):1361–1368
50. Yang Z, Covey MV, Bitel CL, Ni L, Jonakait GM, Levison SW. Sustained neocortical neurogenesis after neonatal hypoxic/ischemic injury. *Ann Neurol*. 2007;61(3):199–208
51. Gonzalez FF, Larpthaveesarp A, McQuillen P, et al. Erythropoietin increases neurogenesis and oligodendrogliosis of subventricular zone precursor cells after neonatal stroke. *Stroke*. 2013;44(3):753–758
52. Gonzalez FF, McQuillen P, Mu D, et al. Erythropoietin enhances long-term neuroprotection and neurogenesis in neonatal stroke. *Dev Neurosci*. 2007;29(4-5):321–330
53. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. *Arch Pediatr Adolesc Med*. 2012;166(6):558–566
54. Shah PS. Hypothermia: a systematic review and meta-analysis of clinical trials. *Semin Fetal Neonatal Med*. 2010;15(5):238–246
55. Cilio MR, Ferriero DM. Synergistic neuroprotective therapies with hypothermia. *Semin Fetal Neonatal Med*. 2010;15(5):293–298

56. Salmeen KE, Jelin AC, Thiet MP. Perinatal neuroprotection. *F1000Prime Rep*. January 2014;6:6

57. Azzopardi D, Robertson NJ, Bainbridge A, et al. Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial. *Lancet Neurol*. 2015;15(2):145–153

58. Baserga MC, Beachy JC, Roberts JK, et al. Darbepoetin administration to neonates undergoing cooling for encephalopathy: a safety and pharmacokinetic trial. *Pediatr Res*. 2015;78(3):315–322

59. Benders MJ, van der Aa NE, Roks M, et al Feasibility and safety of erythropoietin for neuroprotection after perinatal arterial ischemic stroke. *J Pediatr*. 2014;164(3):481–486

60. Andropoulos DB, Brady K, Easley RB, et al. Erythropoietin neuroprotection in neonatal cardiac surgery: a phase I/II safety and efficacy trial. *J Thorac Cardiovasc Surg*. 2013;146(1):124–131

61. Leuchter RH, Gui L, Poncet A, et al. Association between early administration of high-dose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age. *JAMA*. 2014;312(8):817–824

62. Ohls RK, Kamath-Rayne BD, Christensen RD, et al. Cognitive outcomes of preterm infants randomized to darbepoetin, erythropoietin, or placebo. *Pediatrics*. 2014;133(6):1023–1030

63. McAdams RM, McPherson RJ, Mayock DE, Juul SE. Outcomes of extremely low birth weight infants given early high-dose erythropoietin. *J Perinatol*. 2013;33(3):226–230

64. Ohls RK, Cannon DC, Phillips J, et al. Preschool assessment of preterm infants treated with darbepoetin and erythropoietin. *Pediatrics*. 2016;137(3):e20153859

65. Ferriero DM, Miller SP. Imaging selective vulnerability in the developing nervous system. *J Anat*. 2010;217(4):429–435

66. Rutherford M, Ramenghi LA, Edwards AD, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. *Lancet Neurol*. 2010;9(1):39–45

67. Cheong JL, Coleman L, Hunt RW, et al; Infant Cooling Evaluation Collaboration. Prognostic utility of magnetic resonance imaging in neonatal hypoxic-ischemic encephalopathy: substudy of a randomized trial. *Arch Pediatr Adolesc Med*. 2012;166(7):634–640

68. Wu YW, Gonzalez FF. Erythropoietin: a novel therapy for hypoxic-ischaemic encephalopathy? *Dev Med Child Neurol*. 2015;57(suppl 3):34–39

69. Iwai M, Stetler RA, Xing J, et al. Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. *Stroke*. 2010;41(5):1032–1037

High-Dose Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy: A Phase II Trial

Yvonne W. Wu, Amit M. Mathur, Taeun Chang, Robert C. McKinstry, Sarah B. Mulkey, Dennis E. Mayock, Krisa P. Van Meurs, Elizabeth E. Rogers, Fernando F. Gonzalez, Bryan A. Comstock, Sandra E. Juul, Michael E. Msall, Sonia L. Bonifacio, Hannah C. Glass, An N. Massaro, Lawrence Dong, Katherine W. Tan, Patrick J. Heagerty and Roberta A. Ballard

Pediatrics 2016;137;
DOI: 10.1542/peds.2016-0191 originally published online May 2, 2016;

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

High-Dose Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy: A Phase II Trial

Yvonne W. Wu, Amit M. Mathur, Taeun Chang, Robert C. McKinstry, Sarah B. Mulkey, Dennis E. Mayock, Krisa P. Van Meurs, Elizabeth E. Rogers, Fernando F. Gonzalez, Bryan A. Comstock, Sandra E. Juul, Michael E. Msall, Sonia L. Bonifacio, Hannah C. Glass, An N. Massaro, Lawrence Dong, Katherine W. Tan, Patrick J. Heagerty and Roberta A. Ballard

Pediatrics 2016;137;
DOI: 10.1542/peds.2016-0191 originally published online May 2, 2016;