International Journal of Clinical Images and Medical Reviews

Review Article

Brain MRI Outcomes Following Darbepoetin Administration in Newborns Undergoing Hypothermia for Hypoxic-ischemic Encephalopathy (DANCE trial)

Mariana Baserga[1*]; Robert C McKinstry[2]; Betsy Ostrander[3]

[1]Division of Neonatology, Department of Pediatrics, University of Utah, Salt Lake City, Utah, USA.

[2]Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO, USA.

[3]Division of Neurology, Department of Pediatrics, University of Utah, Salt Lake City, Utah, USA.

Abstract

Background: Therapeutic hypothermia improves survival and neurodevelopmental outcomes in neonates with hypoxic-ischemic encephalopathy (HIE). However, significant morbidity and mortality in this population remain unacceptably high. Erythropoietin and the longer-acting synthetic molecule Darbepoetin (Darbe) appear to be neuroprotective in preclinical and clinical trials. Advancements in magnetic resonance imaging (MRI) techniques have improved our ability to evaluate the timing, extent and severity of injury and have become useful in counseling.

Objective: To assess Global Injury Score (GIS) on brain MRI in infants with HIE treated with Darbe as adjunctive therapy to cooling for HIE (DANCE trial).

Methods: Thirty infants (n=10/arm) ≥36 weeks gestation undergoing cooling for HIE were randomized to receive placebo or Darbe. First dose was given within 12 hours of birth, 2nd dose 7 days later. Brain MRIs were scored according to the GIS system by a single neuroradiologist blinded to the treatment group.

Results: Brain MRIs were performed on 27/30 infants between 3-23 days of life (most scans performed at 4-7 days). A larger percentage of patients in the Darbe group had severe encephalopathy at presentation (40 vs. 20%), yet more Darbe-treated patients had either normal or mildly abnormal MRI compared to placebo infants (78 vs. 33%, respectively). The incidence of moderate or severe MRI injury was twice as high in the placebo group compared to the Darbe groups (44 vs 22%).

Conclusions: The majority of infants in each treatment group had normal or only mild injury on MRI. Infants who received Darbe had more severe encephalopathy at presentation yet were less likely to have moderate or severe injury on MRI. Importantly, no cerebral thrombosis was detected which suggests that Darbe does not increase risk of thromboembolism. (NCT01471015; 11/2011).

Keywords: Neonatal encephalopathy; therapeutic hypothermia; erythropoietin; darbepoetin; mri; mri injury scores.

Abbreviations: HIE: Hypoxic-Ischemic Encephalopathy; TH: Therapeutic Hypothermia; GIS: Global Injury Score; DANCE: Darbe Administration In Newborns Undergoing Cooling For Encephalopathy; ESAs: Erythropoiesis Stimulating Agents; Epo: Erythropoietin; Darbe: Darbepoetin Alpha; MRI: Magnetic Resonance Imaging; MRS: Magnetic Resonance Spectroscopy; DWI: Diffusion-Weighted Magnetic Resonance Imaging.

Introduction

Neonatal encephalopathy due to hypoxic-ischemic encephalopathy (HIE) is an important worldwide cause of mortality and morbidity with an incidence of 1 to 6 per 1,000 live births [1]. HIE occurs secondary to inadequate brain blood and oxygen supply and can result in focal or diffuse injury [2]. In spite of the use of therapeutic hypothermia (TH) as standard of care for infants with HIE, recent data demonstrated that up to 30% of treated infants either died or had moderate to severe neurologic disabilities including long-term motor and cognitive dysfunction [3]. Several ongoing clinical trials are currently studying potential neuroprotective therapies to further reduce brain injury and impairment in this population. Among these adjunctive therapies, Erythropoiesis Stimulating Agents (ESAs) have been demonstrated to have anti-apoptotic, antioxidant, and anti-inflammatory properties as well as to support neuronal repair and regeneration in preclinical

***Corresponding Author:** Mariana Baserga, Division of Neonatology, Department of Pediatrics, University of Utah School of Medicine; 295 Chipeta Way, Salt Lake City, UT 84108 United States. Phone: 801-581-7052.
Email: Mariana.Baserga@hsc.utah.edu

Received: Dec 21, 2021 Accepted: Jan 20, 2022 Published: Jan 27, 2022

Citation: Mariana Baserga. Brain MRI Outcomes Following Darbepoetin Administration in Newborns Undergoing Hypothermia for Hypoxic-ischemic Encephalopathy (DANCE trial) Int Clin Img and Med Rew. 2022; 1(1): 1027.

studies [4]. The purpose of the "Darbe Administration in Newborns undergoing Cooling for Encephalopathy (DANCE)" trial was to evaluate the pharmacokinetics and safety of one of these agents, Darbepoetin alpha (Darbe) administered as adjunctive therapy to TH in infants ≥ 36 weeks gestational age with moderate to severe HIE. Darbe given IV in the first 12 hours of life (10 mg/kg/dose) and repeated at 1 week of life in infants undergoing TH for moderate/severe HIE had a pharmacokinetic profile sufficient for weekly administration, allowing for a sustained therapeutic concentration with a longer dosing interval as compared to Erythropoietin (Epo). Infants in the interventional arm had a similar safety profile to placebo [5, 6]. In recent years advancements in brain magnetic resonance imaging (MRI) techniques have helped our ability to evaluate the timing, extent and severity of injury and have become beneficial in counseling families [7, 8]. As part of the standard of care in the management of infants with moderate or severe HIE, a brain MRI was routinely obtained ideally between 4 and 7 days of life post rewarming in the infants that participated in the DANCE trial. The objective of the present study was to assess the Global Injury Score (GIS) system used at Washington University on brain MRI performed in infants with HIE that had received Darbe versus placebo as adjunctive therapy to TH as participants of the DANCE trial [5, 9].

Methods

DANCE was a randomized, placebo-controlled, blinded, multi-dose pharmacokinetic and safety trial. The study inclusion and exclusion criteria and primary outcome results have been published [5]. In brief, the trial included 30 infants (n = 10 in each arm) >36 weeks gestation with moderate to severe HIE as defined by NICHD criteria, [10] and that had received TH by 6 hours of life. Eight centers participated: University of Utah Hospital; Primary Children's Hospital; Intermountain Medical Center; Seattle Children's Hospital; University of New Mexico Children's Hospital and Presbyterian Hospital; Monroe Carell Jr Children's Hospital at Vanderbilt; and McKay Dee Hospital-Intermountain Healthcare [5]. The study received institutional review board approval at the 8 participating hospitals and was registered with clinicaltrials.gov (identifier NCT01471015) and the US Food and Drug Administration (FDA) (Investigational New Drug 113284). A centralized computer randomization website (The University of Utah Data Coordinating and Analysis Center, Salt Lake City, UT) was used to randomize infants to receive either Darbe (Aranesp, Amgen, Thousand Oaks, CA) low dose (2 μg/kg IV); Darbe high dose (10 μg/kg IV); or placebo (normal saline) following informed consent. Treatment was blinded to all caregivers and infants completed 72 hours of TH after which they were rewarmed as per each site cooling protocol. The second study dose was given at 7 days of life (normothermia condition). Adverse events included thrombotic/vascular events, polycythemia, neutropenia, hypotension and/or hypertension, secondary infections, and abnormal hepatic/renal function obtained the first 14 days of life. Clinical outcomes also included the use of seizure medications and feeding status at the time of discharge home. The pharmacokinetics results have also been previously published [6].

Neuroimaging

Informed consent to use the MRI data for this present study was obtained for all participants included in the DANCE study. After obtaining consent, brain MRI scans performed as part of routine clinical care were deidentified and transferred to Washington University, Saint Louis where Dr. Robert McKinstry (Director of Neuroimaging Core) provided centralized blinded interpretation using the Global Injury Score (GIS) system. The GIS scale is a validated, standardized qualitative injury scoring system that grades both anatomic and diffusion scans [11]. The MRI GIS system used in the present study was an adaptation of a previously published system developed at Washington University and that was specifically weighted for posterior limb of the internal capsule (PLIC) and deep nuclear gray matter injuries [9]. These regions were scored in an independent fashion for signal abnormality in each hemisphere on T1-weighted (T1W), T2-weighted (T2W), and diffusion-weighted imaging (DWI) sequences totaling a 6-part score (left and right T1W, T2W, and DWI). A scale of 1 to 4 (except the brainstem, scaled 1 to 3 due to its small size) was used to score all regions. The higher scores represented greater severity of injury (1 = normal; 2 = mild focal abnormality comprising <25% of the region; 3 = moderate multifocal abnormality comprising 25%–50% of the region; 4 = severe widespread abnormality comprising >50% of the region). Component scores, including a white matter score (WMS), cortical score (CS), and cerebellum score (CbS) were developed using the regions graded with the above scale (range 6–24). The brain stem score (BSS) ranged from 6 to 18. The scores from the thalamus, caudate, PLIC, and putamen/globus pallidus were summarized into 1 basal ganglia score (BGS, range 24–96). The final GIS was then calculated as the summation of the 5 subscores (range 48–186): injury was classified as mild if the GIS was between 49 and 59, moderate if between 60 and 80, and severe if ≥81. BGS was purposely more heavily weighted in the GIS because of the severity of neurodevelopmental outcomes following injury to this brain region. The MRI scoring data/entry form is presented in (**Figure 1**). Most all centers used 1.5 T at the time the DANCE trial was carried out. Two centers had a 3T scanner. Each institution utilized their own MRI protocol.

Results

Twenty-nine infants received whole body cooling and one received head cooling. Demographic results were previously published [5] and are included in the present manuscript to help with interpretation of the MRI scoring results. (**Tables 1** and **2**) depict maternal, perinatal, and neonatal clinical characteristics that were similar in the 3 groups. (Table 3) shows the level of encephalopathy at the time of screening for the trial that was as follows: 20 infants (67%) had moderate encephalopathy (placebo= 8; low-dose Darbe= 7; high-dose Darbe= 5) and 10 infants had severe encephalopathy (placebo= 2; low-dose Darbe= 3; high-dose Darbe group= 5). Six infants had seizures at the time of screening: 2 in the placebo group, 3 in the low-dose Darbe group, and one in the high-dose Darbe group. At time of hospital discharge the use of anticonvulsants for seizures was similar between the study groups, however, feeding difficulties (Gavage feedings/G tube) were more prevalent in the placebo group (3/10) versus the Darbe group (4/18). Adverse events have also been reported. There were 2 deaths that were associated with multiorgan failure and not attributed to study participation. No other serious adverse events were reported

Neuroimaging Core – Standardized Scoring System CRF

Reader: Robert McKinstry, MD Date: ____ /____ /_____ (mm/dd/yyyy)

Study Subject ID: __ __ - __ __ (2 digit site code - 2 digit subject number) MRS: yes no

	T1	T2	DWI	
DNGM	**Left Right**	**Left Right**	**Left Right**	
caudate	1→4 1→4	1→4 1→4	1→4 1→4	
put/glob**	1→4 1→4	1→4 1→4	1→4 1→4	
thalamus	1→4 1→4	1→4 1→4	1→4 1→4	**Σ= BGS**
PLIC	1→4 1→4	1→4 1→4	1→4 1→4	
WM	1→4 1→4	1→4 1→4	1→4 1→4	**Σ= WMS**
Cortex	1→4 1→4	1→4 1→4	1→4 1→4	**Σ= CS**
Brainstem	1→3 1→3	1→3 1→3	1→3 1→3	**Σ= BSS**
Cerebellum	1→4 1→4	1→4 1→4	1→4 1→4	**Σ= CbS**
			Σ= DS	**Σ= GS**

1→4:
1= no abnomality
2= mild abnormality: focal abnormal signal (< 25%*)
3= moderate abnormality: multifocal abnormal signal (25-50%) 4= severe abnormality: widespread abnormal signal (>50%) 1→3:
1= no abnomality
2= moderate abnormality: focal abnormal signal
3= severe abnormality: widespread abnormal signal
* : % of the analyzed structure
** : put/glob= putamen/ globus pallidus

Notes:_____

Radiology Reader:

Imaging Coordinator:

Date Data Returned

Figure 1: The MRI scoring data entry form used in the study.
MRS= Magnetic Resonance Spectroscopy; DNGM=Deep Nuclear Gray Matter; Put/Glob= Putamen/Globus Pallidus; PLIC= Posterior Limb Of The Internal Capsule; WM= White Matter; BGS= Basal Ganglia Score; DWI: Diffusionweighted Magnetic Resonance Imaging; CS= Cortical Score; BSS= Brain Stem Score; CBS= Cerebellum Score; DS= Diffusion Score; GS= Global Score.

and comorbidities were similar between all three study groups (hypotension, altered renal function, and pulmonary hypertension) [5].

MRI results and Injury Scores

A total of 27 MRI studies (one per subject, 9 in each study group) were performed at a median of 12 days (range 3–23) though 19/27 of the scans were performed between days 4-7. The remainder 8 scans were obtained at a later time in 4 placebo infants and in 4 Darbe treated infants. Two patients died before a brain MRI could be performed. One family (highdose Darbe group) could not be reached to obtain consent to deidentify and transfer the MRI to be scored by our central reader. The final GIS scores are shown by study group in (**Table 3**).

After reviewing the MRI GIS results, the 2 Darbe treatment arms were combined into one group since the 2 groups had similar scores. Most infants had either a normal or mildly affected MRI. A subdural hematoma was observed in 7 babies but none had evidence of sinovenous thrombosis. A larger percentage of patients in the Darbe group had severe encephalopathy at presentation (40 vs. 20%), yet more Darbe treated patients had either normal or mildly abnormal MRI compared to placebo infants (78 vs. 33%, respectively). The incidence of moderate or severe MRI injury was twice as high in the placebo group compared to the Darbe groups (44 vs 22%).

Discussion

In the present study we assessed the GIS scoring system developed at Washington University on brain MRI performed in infants with neonatal encephalopathy secondary to HIE that were treated with Darbe versus placebo as adjunctive therapy to hypothermia in the DANCE

trial [5, 9]. Our main observation was that in spite of worse initial encephalopathy, infants who received Darbe as an adjunctive therapy to TH were less likely to have moderate or severe injury on MRI. The score used in the present paper has the advantage of including DWI and can, therefore, be used in the first week of life, a period during which additional neuroprotective therapies can be initiated as well as when important clinical decisions may need to be made. Importantly, no cerebral thrombosis was detected which suggests that Darbe does not increase risk of thromboembolism.

The information obtained from MRI scans integrated into clinical practice can be useful in parental counseling and to support discussions regarding long-term outcomes [12]. In particular, worse neurodevelopmental outcomes have been observed following injuries to deep nuclear gray matter and the posterior limb of the internal capsule [13]. To this end, different MRI Injury scoring systems have been advocated in the past to standardize results in an attempt to assist with

Table 1-Maternal characteristics	Placebo (n=10)	Low Dose Darbe 2 μg/kg (n=10)	High Dose Darbe 10 μg/kg (n=10)	Total All Patients (n=30)
Maternal				
Maternal Age- years [a]	29 (18-35)	30 (20-35)	29 (21-33)	
Complications of Pregnancy Preeclampsia-n (%)	3	0	0	3 (10)
Rupture of membranes>18H-n (%)	0	2	0	2 (7)
Antepartum Hemorrhage-n (%)	2	0	1	3 (10)
Intrapartum Complications Fetal Heart Rate Decelerations-n (%)	8	8	10	26 (87)
Placental Problems: Abruption, Previa, -n (%)	3	2	4	9 (30)
Uterine Rupture-n (%)	1	0	3	4 (13)
Cord Prolapse-n (%)	0	2	0	2 (7)
Vacuum/Forceps Delivery-n (%)	2	2	1	5 (17)

[a]Median (range)

Table 2- Neonatal characteristics	Placebo (n=10)	Low Dose Darbe 2 μg/kg (n=10)	High Dose Darbe 10 μg/kg (n=10)	Total All Patients (n=30)
Neonatal				
Age At Randomization- hours [a]	6 (5-9)	8 (2-11)	7 (2-10)	7 (2-11)
Transferred From Birth Hospital- n (%)	5	4	6	15 (50)
Gestational Age- weeks [a]	38 (36-41)	39 (36-41)	39 (37-40)	39 (36-41)
Birth Weight (g) [b]	3093 ± 388	2892 ± 440	3042 ± 390	3009 ± 401
Male Sex-n (%)	4	5	6	15 (50)
Apgar Score (≤5)-n (%) [a] 5 Minutes	8	8	8	24 (80)
10 Minutes	6	8	7	21 (70)
Positive pressure/intubation in delivery room- n (%)	8	5	10	23 (77)
Chest compressions at delivery- n (%)	7	4	6	17 (57)
Epinephrine- n (%)	4	2	4	10 (33)
Cord blood (arterial or venous) [a] pH	7.00 (6.60-7.3)	7.00 (6.70-7.20)	7.20 (6.80-7.30)	7.00 (6.60-7.30)
Base Deficit-mEq/liter	18 (7-27)	13 (7-28)	11 (5-26)	14 (5-28)
Blood Gas within 60 minutes of age [a] pH	7.20 (6.60-7.30)	7.00 (6.60-7.20)	7.10 (6.70-7.30)	7.00 (6.60-7.30)
Base Deficit-mEq/liter	14 (4-30)	15 (12-21)	17 (8-34)	15 (4-34)
Seizures [c]	2	3	1	6 (20)

[a]Median (range); [b]Mean ± SD, [c]Data are for these characteristics at time of randomization.

Table 3: Encephalopathy Level and MRI scores by treatment allocatio.

Encephalopathy Level (at time of Randomization)	Placebo (n=9)	Darbe 2 or 10 µg/kg (n=18)	All Patients (n=27)
Moderate Encephalopathy	8	11	19
Severe Encephalopathy	1	7	8
MRI GIS			
Normal (score =48)	3	7	10
Mild Injury (score= 49-59)	2	7	9
Moderate Injury (score=60-80)	4	3	7
Severe Injury (score>81)	0	1	1

prognosis and counseling [9, 14-16]. The literature investigating the effects of ESAs upon MRI injury following HIE or prematurity is limited. The Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes (NEATO) study was a multicenter, double-blinded controlled trial in term newborns with moderate to severe HIE that received five doses of erythropoietin (Epo) (1000 U/kg IV) versus placebo for 7 days as adjunctive therapy to TH [17]. Similar to the DANCE trial findings, the NEATO study also showed that the administration of Epo was associated with reduced severity of brain injury on neonatal MRI performed at 4 to 7 days of age, specifically in the subcortical region. Two central reviewers who were blinded to treatment allocation, independently scored brain MRI findings using the Global brain injury scale that established injury severity in 8 brain regions in each hemisphere (cortex, brainstem, thalamus, caudate, putamen/globus pallidus, PLIC, white matter, and cerebellum). Infants in the Epo arm had significantly lower global brain injury scores than those in the placebo group and fewer Epo-treated infants had moderate to severe brain injury on MRI. Alterations in the basal ganglia, thalamus, and/or PLIC and cerebellum were also significantly less common in the Epo than in the placebo group. A large phase III trial (HEAL) to determine whether Epo administration as adjunctive therapy to TH in infants with HIE is underway. One of the components of this large trial is MRI assessment of injury. A standardized 3T brain MRI and MRS was performed following rewarming (between 4 and 6 days of life) and imaging data acquisition was harmonized across different MRI makes and models. Furthermore, a standardized HEAL MRI and MRS protocol using an iterative quality control process was implemented. Deidentified brain MRI studies are being read centrally by 2 independent readers blinded to treatment arm. The GIS system from Washington University is also being used to establish severity and location of brain injury.

Two studies have assessed MRI outcomes in premature babies that received ESAs for neuroprotection. In a large multicenter placebo-controlled randomized trial preterm infants (2632 weeks GA), there were no significant differences in neurodevelopmental outcomes at 2 years between the interventional arm (early high-dose Epo) and placebo group [18]. However, subgroup analyses found that Epo treated infants had improved white matter development in the major white matter tracts and an increase of local structural connectivity strengths while demonstrating reduced brain injury [19, 20]. Therefore, follow up of this cohort into later childhood would be crucial. The second trial performed MRI scans at 3.5 to 4 years of age on former preterm infants treated with Epo, Darbe or placebo from the time of delivery through 35 weeks CGA, and compared them to healthy term controls. There was no significant effect of ESAs on surface area, cortical thickness or fractional anisotropy. However, there was a greater increase in fractional anisotropy among ESA treated girls. Significant correlations between fractional anisotropy and full scale IQ and verbal IQ were found in group analysis [21].

MRI injury scoring scales published in the literature include the National Institutes for Child Health and Development system (NICHD; Shankaran), the Barkovich system, the Rutherford system, and most recently, the MRI injury scoring system published by the Washington University group (Trivedi et al, 2017) and by Weeke et al (2018) [9, 14, 15, 22, 23]. Most initially published MRI scoring systems that were associated with long term outcome were designed to be performed using T1- and T2-weighted images only and therefore scored MRIs were obtained in the second week of life [12, 14]. The advantage of the most recent scoring systems is that they incorporate DWI sequences in addition to T1, T2 sequences [9, 22]. DWI is a useful MRI sequence with the capability of detecting injury as early as 24–72 h of life. The combination of DWI sequences with T1-W, T2-W in the first 2- to 5-day window can help determine the extent and timing of HIE injury [24, 25]. Furthermore, as compared with earlier systems, the University of Washington and Weeke scoring tools separately weigh subcortical injury in the posterior limb of the internal capsule and deep nuclear gray matter [9, 22]. This injury assessment is important based on recent literature demonstrating the association of deep nuclear gray matter and

PLIC injuries with poor neurodevelopmental outcomes [8, 26]. Lastly, an advantage of the GIS scoring system presented in this study is the point-by-point entry form which can be used by less experienced MRI readers.

The main limitation of our study is the small number of participants as well as the lack of long term outcomes. Nonetheless, other reports have consistently demonstrated that a qualitative MRI scoring system can be used as a significant predictor of neurodevelopmental outcomes following HIE [9, 22, 23]. In a single-center study that included 57 infants with moderate to severe HIE treated with TH, Trivedi et al validated a previous version of the Washington University GIS MRI scoring system with neurodevelopmental outcomes at 18 and 24 months. Higher MRI injury scores and grades were significantly associated with lower performance scores across all three domains of the Bayley-III. Similarly, Weeke at al developed a comprehensive MRI scoring system that had good predictive value in 2 independent, international cohorts of 173 infants treated with TH. The authors concluded that injury to the deep gray matter area was an independent predictor of poor neurodevelopmental outcomes both at 2 years of age and at school age [22].

A second limitation of this study was that even though most MRI scans were performed between 4-7 days of life, there were a few performed at a later stage. MRIs were performed following the standard of care in the participating centers and each institution followed their own MRI protocols. Moreover, most participating centers (5) at the time of the trial (2013-2015) still utilized 1.5T MRI scanners and the remainder 2 had 3T scanners. In spite of these inter center variabilities, the MRIs evaluated by Dr. McKinstry were deemed of good technical quality to be included in the analysis using the GIS scoring system.

A second MRI scan was not available for scoring. In 2014, the ACOG Task Force on Neonatal Encephalopathy published an executive summary outlining recommendations for neuroimaging following neonatal encephalopathy. They recommend 2 MRI scans if possible: the first one obtained early in the neonatal course (24–96 h) used to delineate the timing of perinatal injury, and the second between 7 and 21 days of life, used to establish the extent of injury with an ideal goal of obtaining a second MRI scan on day of life 10 [24]. In clinical practice, the timing of the MRI scan/s typically depends on the clinical stability of the infant, access to MRI and information expected to be obtained from the MRI [27]. Lastly, a third limitation of this study was that the MRI scans were reviewed and scored by just one reader. Ideally, 2 independent readers would review the scans and any discrepancies would be resolved by consensus review. The consensus review typically results in identification of additional injury when there is discrepancy between the 2 readers. As such, a single reader study may have lower overall GIS but there is no reason to suspect that the GIS scores would differentially impact the two treatment arms of the study. Lastly, prior MRI scoring studies found high inter-rater reliability [9, 22].

In summary, in this observational study infants who received Darbe as adjunctive therapy to TH had more severe encephalopathy based on neurological examination at presentation yet were less likely to have moderate or severe injury on MRI. Despite the limitations described earlier, this study provides information regarding feasibility and reproducibility of the Washington University GIS MRI scoring system. Early knowledge of the pattern, extent and severity of brain injury following HIE has the potential to assist the clinical team with early prognostication, counseling and timely intervention.

Declarations

Funding: This study was supported by Thrasher Research Fund (Salt Lake City, UT) through an E.W. "AL" Thrasher Award to M.C.Baserga-(10026771-F1).

Conflicts of interest/Competing interests: R.C. McKinstry is a member of the medical advisory board for Nous Imaging, Inc. This author also received travel, meals and lodging from Siemens Healthineers, and meals from Philips Healthcare.

Availability of data and material: All data and materials as well as software application support their published claims and comply with field standards. Datasets are available upon reasonable request. The corresponding author Mariana Baserga can be contacted if someone requests the data.

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mariana Baserga, Betsy Ostrander and Robert McKinstry. The first draft of the manuscript was written by Mariana Baserga and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and Consent to participate: This study was approved by the institutional research ethics committee at each participating institution (University of Utah Hospital; Primary Children's Hospital; Intermountain Medical Center; Monroe Carell Jr Children's Hospital at Vanderbilt; University of New Mexico Children's Hospital and Presbyterian Hospital; Seattle Children's Hospital; and McKay Dee HospitalIntermountain Healthcare). We certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained for this study.

Acknowledgments: The coauthors of the DANCE trial: Beachy, J. C.; Roberts, J. K.; Ward, R. M.; DiGeronimo, R. J.; Walsh, W. F.; Ohls, R. K.; Anderson, J.; Mayock, D. E.; Juul, S. E.; Christensen, R. D.;

Loertscher, M. C.; Stockmann, C.; Sherwin, C. M.; Spigarelli, M. G.; Yoder, B. A.

References

1. Chau V, Poskitt KJ, Dunham CP, Hendson G, Miller SP. Magnetic resonance imaging in the encephalopathic term newborn. Curr Pediatr Rev. 2014; 10(1):28-36. [DOI:10.2174/1573396310011404081203 36].

2. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy.

Early Hum Dev. Jun 2010; 86(6):329-38. [DOI:10.1016/j.earlhumdev.2010.05.010].

3. Shankaran S, Laptook AR, Pappas A, et al. Effect of Depth and Duration of Cooling on Death or Disability at Age 18 Months Among Neonates With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. JAMA. Jul 4 2017; 318(1):57-67. [DOI:10.1001/jama.2017.7218].

4. Grasso G, Graziano F, Sfacteria A, et al. Neuroprotective effect of erythropoietin and darbepoetin alfa after experimental intracerebral hemorrhage. Neurosurgery. Oct 2009; 65(4):763-9; discussion 769-70. [DOI:10.1227/01.NEU.0000347475.73347.5F].

5. Baserga MC, Beachy JC, Roberts JK, et al. Darbepoetin administration to neonates undergoing cooling for encephalopathy: a safety and pharmacokinetic trial. Pediatr Res. Sep 2015; 78(3):315-22. [DOI:10.1038/pr.2015.101].

6. Roberts JK, Stockmann C, Ward RM, et al. Population Pharmacokinetics of Darbepoetin Alfa in Conjunction with Hypothermia for the Treatment of Neonatal Hypoxic-Ischemic Encephalopathy. Clin Pharmacokinet. Dec 2015; 54(12):1237-44. [DOI:10.1007/s40262-0150286-y].

7. Cowan F, Rutherford M, Groenendaal F, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. Lancet. Mar 1 2003; 361(9359):736-42. [DOI:10.1016/S0140-6736(03)12658-X].

8. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. J Pediatr. Apr 2005; 146(4):453-60. [DOI:10.1016/j.jpeds.2004.12.026].

9. Trivedi SB, Vesoulis ZA, Rao R, et al. A validated clinical MRI injury scoring system in neonatal hypoxic-ischemic encephalopathy. Pediatric radiology. Oct 2017; 47(11):1491-1499. [DOI:10.1007/s00247-017-3893-y].

10.Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med. Oct 13 2005; 353(15):1574-84. [DOI:10.1056/NEJMcps050929].

11. Bednarek N, Mathur A, Inder T, Wilkinson J, Neil J, Shimony J. Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy. Neurology. May 1 2012; 78(18):1420-7. [DOI:10.1212/WNL.0b013e318253d589].

12. 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. Jan 2010; 9(1):39-45. [DOI:10.1016/S14744422(09)70295-9].

13. Hunt RW, Neil JJ, Coleman LT, Kean MJ, Inder TE. Apparent diffusion coefficient in the posterior limb of the internal capsule predicts outcome after perinatal asphyxia. Pediatrics. Oct 2004; 114(4):999-1003. [DOI:10.1542/peds.2003-0935-L].

14. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. AJNR Am J Neuroradiol. Jan 1998; 19(1):143-9.

15. Rutherford M, Srinivasan L, Dyet L, et al. Magnetic resonance imaging in perinatal brain injury: clinical presentation, lesions and outcome. Pediatr Radiol. Jul 2006; 36(7):582-92. [DOI:10.1007/s00247-006-0164-8].

16. Azzopardi D, Edwards AD. Magnetic resonance biomarkers of neuroprotective effects in infants with hypoxic ischemic encephalopathy. Seminars in fetal & neonatal medicine. Oct 2010; 15(5):261-9. [DOI:10.1016/j.siny.2010.03.001].

17. Wu YW, Mathur AM, Chang T, et al. High-Dose Erythropoietin and Hypothermia for Hypoxic-Ischemic Encephalopathy: A Phase II Trial. Pediatrics. Jun 2016; 137(6). [DOI:10.1542/peds.2016-0191].

18. Natalucci G, Latal B, Koller B, et al. Effect of Early Prophylactic High-Dose Recombinant Human Erythropoietin in Very Preterm Infants on Neurodevelopmental Outcome at 2 Years: A Randomized Clinical Trial. JAMA. May 17 2016; 315(19):2079-85. [DOI:10.1001/jama.2016.5504].

19. Leuchter RH, Gui L, Poncet A, et al. Association between early administration of highdose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age. JAMA. Aug 27 2014; 312(8):817-24. [DOI:10.1001/jama.2014.9645].

20. Jakab A, Ruegger C, Bucher HU, et al. Network based statistics reveals trophic and neuroprotective effect of early high dose erythropoetin on brain connectivity in very preterm infants. Neuroimage Clin. 2019; 22:101806. [DOI:10.1016/j.nicl.2019.101806].

21. Phillips J, Yeo RA, Caprihan A, et al. Neuroimaging in former preterm children who received erythropoiesis stimulating agents. Pediatr Res. Oct 2017; 82(4):685-690. [DOI:10.1038/pr.2017.130].

22. Weeke LC, Groenendaal F, Mudigonda K, et al. A Novel Magnetic Resonance Imaging Score Predicts Neurodevelopmental Outcome After Perinatal Asphyxia and Therapeutic Hypothermia. J Pediatr. Jan 2018; 192:33-40 e2. [DOI:10.1016/j.jpeds.2017.09.043].

23. Shankaran S, Barnes PD, Hintz SR, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. Archives of disease in childhood Fetal and neonatal edition. Nov 2012; 97(6):F398-404. [DOI:10.1136/archdischild-2011-301524].

24. Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. Report of the American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. Obstet Gynecol. Apr 2014; 123(4):896-901. [DOI:10.1097/01.AOG.0000445580.65983.d2].

25. Gano D, Chau V, Poskitt KJ, et al. Evolution of pattern of injury and quantitative MRI on days 1 and 3 in term newborns with hypoxic-ischemic encephalopathy. Pediatr Res. Jul 2013; 74(1):82-7.

[DOI:10.1038/pr.2013.69].

26. Barnett A, Mercuri E, Rutherford M, et al. Neurological and perceptual-motor outcome at 5 - 6 years of age in children with neonatal encephalopathy: relationship with neonatal brain MRI. Neuropediatrics. Oct 2002; 33(5):242-8. [DOI:10.1055/s-2002-36737].

27. 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. Mar 2006; 27(3):533-47.