Pediatric RESEARCH

www.nature.com/pr

CLINICAL RESEARCH ARTICLE

Transfusions and neurodevelopmental outcomes in extremely low gestation neonates enrolled in the PENUT Trial: a randomized clinical trial

Phuong T. Vu[1,27], Robin K. Ohls[2], Dennis E. Mayock[3], Kendell R. German[3], Bryan A. Comstock[1], Patrick J. Heagerty[1], Sandra E. Juul[3] and for the PENUT Consortium

BACKGROUND: Outcomes of extremely low gestational age neonates (ELGANs) may be adversely impacted by packed red blood cell (pRBC) transfusions. We investigated the impact of transfusions on neurodevelopmental outcome in the Preterm Erythropoietin (Epo) Neuroprotection (PENUT) Trial population.

METHODS: This is a post hoc analysis of 936 infants 24-0/6 to 27-6/7 weeks' gestation enrolled in the PENUT Trial. Epo 1000 U/kg or placebo was given every 48 h × 6 doses, followed by 400 U/kg or sham injections 3 times a week through 32 weeks postmenstrual age. Six hundred and twenty-eight (315 placebo, 313 Epo) survived and were assessed at 2 years of age. We evaluated associations between BSID-III scores and the number and volume of pRBC transfusions.

RESULTS: Each transfusion was associated with a decrease in mean cognitive score of 0.96 (95% CI of [−1.34, −0.57]), a decrease in mean motor score of 1.51 (−1.91, −1.12), and a decrease in mean language score of 1.10 (−1.54, −0.66). Significant negative associations between BSID-III score and transfusion volume and donor exposure were observed in the placebo group but not in the Epo group.

CONCLUSIONS: Transfusions in ELGANs were associated with worse outcomes. We speculate that strategies to minimize the need for transfusions may improve outcomes.

Pediatric Research (2021) 90:109–116; https://doi.org/10.1038/s41390-020-01273-w

IMPACT:

- Transfusion number, volume, and donor exposure in the neonatal period are associated with worse neurodevelopmental (ND) outcome at 2 years of age, as assessed by the Bayley Infant Scales of Development, Third Edition (BSID-III).
- The impact of neonatal packed red blood cell transfusions on the neurodevelopmental outcome of preterm infants is unknown.
- We speculate that strategies to minimize the need for transfusions may improve neurodevelopmental outcomes.

INTRODUCTION

There is wide practice variation in the application of transfusion guidelines and the indications used to transfuse packed red blood cell (pRBC) in the Neonatal Intensive Care Unit (NICU). Despite this variation, the vast majority of extremely low gestational age neonates (ELGANs, <28 weeks' gestation) receive at least 1 pRBC transfusion, with the median number ranging from 3 to 8 during their hospitalization, the majority of which occur in the first month after birth.[1,2]

A better understanding of the potential risks and benefits associated with pRBC transfusions is needed to help guide appropriate transfusion practices in the NICU. Potential short-term risks include infection, inflammation, transfusion-related lung injury, and transfusion-related necrotizing enterocolitis.[3–8] A potential long-term risk associated with pRBC transfusion includes neurodevelopmental (ND) modulation. In a previous study evaluating erythropoiesis-stimulating agents (ESAs) that utilized a restrictive pRBC transfusion strategy, cognitive scores at 18–22 months were inversely correlated with transfusion volume.[9] However, the long-term effect of anemia vs. pRBC transfusion on ND is unclear, as reports are few and conflicting.[10–12] The recently published "Effects of Transfusion Thresholds on Neurocognitive Outcome of Extremely Low Birth-Weight (ELBW) Infants (ETTNO) Study[13,14] (NCT01393496) showed no difference in outcomes between liberal and restrictive transfusion approaches (N-1013). The ongoing Transfusion of Prematures (TOP) Trial (NCT01702805) will shed further light on this question. However, neither the ETTNO or TOP study addresses the interaction of transfusion with

[1]Department of Biostatistics, University of Washington, Seattle, WA, USA; [2]Department of Pediatrics, University of Utah, Salt Lake City, UT, USA and [3]Department of Pediatrics, University of Washington, Seattle, WA, USA
Correspondence: Sandra E. Juul (sjuul@uw.edu)
[27]Present address: Seattle Children's Research Institute, Seattle, WA, USA
A list of authors and their affiliations appears below the Acknowledgements.

Received: 23 June 2020 Revised: 19 August 2020 Accepted: 2 October 2020
Published online: 11 January 2021

the use of ESAs, and the ETTNO study did not compare outcomes of those infants who received transfusions with those who were transfusion-free.

Using data from the Preterm Erythropoietin Neuroprotection Trial (PENUT; NCT01378273), we now investigate the relationship between the number and volume of transfusions given to ELGANs and their ND outcome at 22–24 months of age. In addition, after adjusting for covariates, we evaluate whether infants who remain transfusion-free have better cognitive outcomes than ELGANS who receive ≥1 pRBC transfusions.

METHODS

Study design and population

The PENUT Trial was a multi-center, randomized, placebo-controlled, double masked study examining the use of erythropoietin (Epo) for neuroprotection in infants born between 24-0/7 and 27-6/7 weeks of gestation. A total of 941 infants at 19 sites across the United States were enrolled between December 2013 and September 2016. Parental consent, enrollment, and initial treatment with study drug occurred within 24 h of birth. Subjects received Epo 1000 U/kg/dose or placebo intravenously every 48 h for 6 doses, followed by Epo 400 U/kg/dose by subcutaneous injection or sham (no actual needle stick) 3 times a week until the subjects completed 32 weeks postmenstrual age (PMA). An investigational New Drug (IND) application was approved for the PENUT Trial by the Food and Drug Administration (IND#12656). The PENUT Trial was registered in ClinicalTrials.gov (NCT01378273) and was approved by each site's Institutional Review Board. Details of eligibility, enrollment, and randomization have been published elsewhere.[15,16] PENUT was powered for the primary outcome of death or ND impairment (NDI) at 2 years. This paper focuses on randomized infants who survived and underwent 2-year ND assessment in compliance with protocol.

Guidelines for iron supplementation were included in the study protocol. When feedings were initiated, a standard iron-containing formula was used if breast milk was unavailable. When subjects reached an enteral intake of 60 mL/kg/day and were at least 7 days of age, 3 mg/kg/day enteral iron was recommended. Iron dosing was increased to 6 mg/kg/day when infants reached an enteral intake of 100 to 120 mL/kg/day. To assess infant iron status, serum ferritin or zinc protoporphyrin-to-heme ratio[17] was to be measured at 14 and 42 days, and iron dosing increased or decreased accordingly. Infants who were not able to tolerate enteral feedings were given maintenance iron parenterally (3 mg/kg/week of iron dextran or iron sucrose, dose adjusted based on iron indices).

As there are no consensus recommendations for pRBC transfusions in critically ill neonates, each site followed their own pRBC transfusion protocol and procedures. We documented the formal transfusion guidelines for sites when available. Transfusion records were considered complete it they included both volume and donor information. Information from infants with no transfusion was also included in the analysis.

In addition to clinical characteristics and demographic information, pRBC transfusion number, volume, and donor exposures were recorded for each subject. Infants without complete 2-year developmental follow-up assessment were excluded from these analyses.

Outcomes

The primary ND outcome of interest was the Bayley Scales of Infant Development Third Edition (BSID-III) evaluated at 22–26 months, which has 3 composite components: cognitive, motor, and language scores. Secondary outcomes included severe NDI, defined as the presence of any one of the following: severe cerebral palsy (CP), (defined as Gross Motor Function Classification System (GMFCS) >2), BSID-III cognitive score <70, or BSID-III motor score <70; and moderate-to-severe NDI, defined as the presence of any one of the following: moderate-to-severe CP (GMFCS ≥2), BSID-III cognitive score <85, or BSID-III motor score <85.[15]

Statistical analysis

Our post hoc statistical analyses included randomized infants who received at least the first dose of study treatment, survived, and underwent 2-year ND assessment. All analyses accounted for potential sibship correlation by using Generalized Estimating Equations (GEE)[18] with robust standard errors.

The primary goal was to evaluate the associations between mean BSID-III component scores and three transfusion variables: the total number of pRBC transfusions, the cumulative transfusion volume, and the number of unique donor exposures. These associations were examined among all participants using GEE models adjusted for treatment group, gestational age at birth (grouped as 24/25-week or 26/27 weeks), recruitment site, and covariates that potentially impact receipt of transfusions. The potential confounding covariates included birth weight, Apgar score at 5 min, suspected or confirmed chorioamnionitis, and the use of ventilator for respiratory support at enrollment. The associations between BSID-III scores and transfusion exposures were further assessed within the 2 treatment arms using GEE models adjusted for gestational age group, recruitment site, and the covariates considered as potential confounders.

In secondary analyses, we used similar GEE models to evaluate associations between BSID-III scores and transfusion status (i.e., transfusions yes/no) for all participants and within each treatment arm. We also evaluated associations between the secondary outcomes of interest and transfusion exposures or transfusion status among all participants using GEE loglinear models, adjusting for treatment group, and gestational age group. Associations were again stratified based on treatment group. We did not adjust for recruitment site and other confounding covariates at baseline in these analyses due to low count of events relative to a large number of sites and the number of covariates.

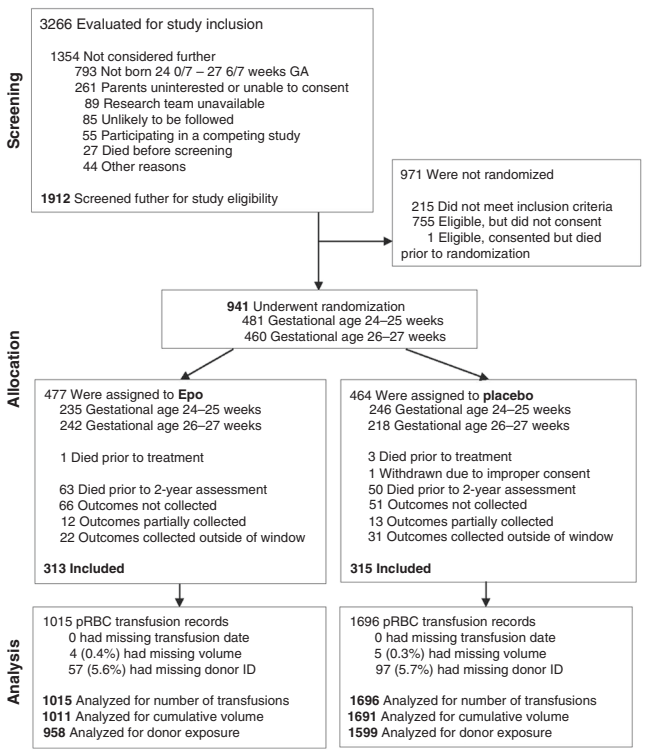


Fig. 1 CONSORT diagram. This figure shows the infants screened, enrolled, randomized to Epo or placebo, and transfusion records analyzed.

Table 1. Maternal demographics and neonatal data of the 628 included infants.

	Placebo ($N = 315$)	Epo ($N = 313$)	p value[a]
Had any red cell transfusions, n (%)	286 (91%)	226 (72%)	<0.01
Maternal demographics			
Age, mean (SD)	28.7 (6.2)	29.7 (6.1)	0.05
Hispanic[b], *n* (%)	71 (23%)	64 (20%)	0.79
Race, *n* (%)			0.31
White	217 (69%)	213 (68%)	
Black	64 (20%)	75 (24%)	
Others, unknown, or unreported	34 (11%)	25 (8%)	
Education[c], *n* (%)			0.16
High school or less	107 (34%)	86 (27%)	
Some college	88 (28%)	107 (34%)	
College degree or greater	88 (28%)	85 (27%)	
Neonatal data at enrollment			
Maternal indications for delivery, *n* (%)	55 (17%)	45 (14%)	0.37
Risk of chorioamnionitis[d], *n* (%)	237 (75%)	238 (76%)	0.90
Pregnancy-induced hypertension, *n* (%)	23 (7%)	22 (7%)	0.78
Prenatal steroids[e], *n* (%)	290 (92%)	284 (91%)	0.45
Prenatal magnesium sulfate[f], *n* (%)	261 (83%)	248 (79%)	0.17
Delivery complications[g], *n* (%)	49 (16%)	55 (18%)	0.55
Cesarean delivery, *n* (%)	210 (67%)	208 (66%)	0.90
Delayed cord clamping[h], *n* (%)	110 (35%)	120 (38%)	0.39
Sex—male, *n* (%)	157 (50%)	162 (52%)	0.62
Birth gestational age, *n* (%)			0.29
24 weeks	78 (25%)	73 (23%)	
25 weeks	84 (27%)	74 (24%)	
26 weeks	81 (26%)	70 (22%)	
27 weeks	72 (23%)	96 (31%)	
Mean (sd)	25.9 (1.1)	26.0 (1.2)	0.22
Multiple gestation, *n* (%)	82 (26%)	81 (26%)	0.94
Birth weight in grams, mean (sd)	802.8 (186.3)	819.4 (191.4)	0.60
Apgar score at 5 min <5[i]	54 (17%)	59 (19%)	0.30

[a]p values were obtained from GEE models evaluating the difference in each baseline characteristic between treatment groups, adjusting for potential sibship correlations, gestational weeks at birth (if applicable), and site grouping.
[b]Ethnicity was unknown or unreported for 1 subject (<1%) in the Epo group.
[c]Maternal education was unknown or unreported for 32 (10%) and 35 (11%) subjects in the placebo and Epo groups, respectively.
[d]Risk of chorioamnionitis included prolonged ruptured of membranes, suspected or confirmed chorioamnionitis, pre-term labor, pyrexia ≥38.1 °C in labor, and antibiotic administration.
[e]Prenatal steroids were unknown for 5 (2%) and 3 (1%) subjects in the placebo and Epo groups, respectively.
[f]Prenatal magnesium sulfate was unknown for 16 (5%) and 7 (2%) subjects in the placebo and Epo groups, respectively.
[g]Delivery complications were defined as the presence of one or more of the following complications during delivery: prolapsed cord, true knot, tear or rupture of cord, placental abruption, twin–twin transfusion, feto-maternal bleeding, ruptured uterus, or traumatic instrument delivery.
[h]Delayed cord clamping was unknown for 88 (28%) placebo and 84 (27%) Epo subjects.
[i]Apgar score at 5 min was missing for 3 (1%) subjects in the Epo group.

In sensitivity analyses, we assessed the primary associations between BSID-III component scores and pRBC exposures for subgroups defined by sex or gestational age (24, 25, 26, and 27 weeks).

All statistical tests were two sided and statistical significance was set at level 0.05. All analyses were post hoc and not adjusted for multiple testing. Statistical analyses were performed using the R statistical software package (version 3.5.1, Vienna, Austria).[19]

RESULTS

The 628 infants (315 placebo and 313 Epo treated) who survived and had 2-year ND outcomes collected per protocol and who were included in these analyses comprised 67% of the total 941 subjects enrolled in the PENUT Trial. There were 2711 complete pRBC transfusion records available across all neonates. Screening, inclusion, and available analysis data are summarized as a CONSORT diagram in Fig. 1. Maternal demographics and neonatal descriptors are shown in Table 1. Of the 628 infants, 29 (9%) in the placebo group and 87 (28%) in the Epo group remained transfusion-free ($p < 0.01$). The 315 infants in the placebo group received a mean of 5.4 (SD = 4.8) transfusions and 83.7 (SD = 87.6) mL cumulative pRBC volume from 2.4 (SD = 2.2) unique donors. In comparison, the 313 infants in the Epo group received a mean of 3.2 (SD = 3.7) transfusions and 46.6 (SD = 61.8) mL cumulative pRBC volume from 1.6 (SD = 1.9)

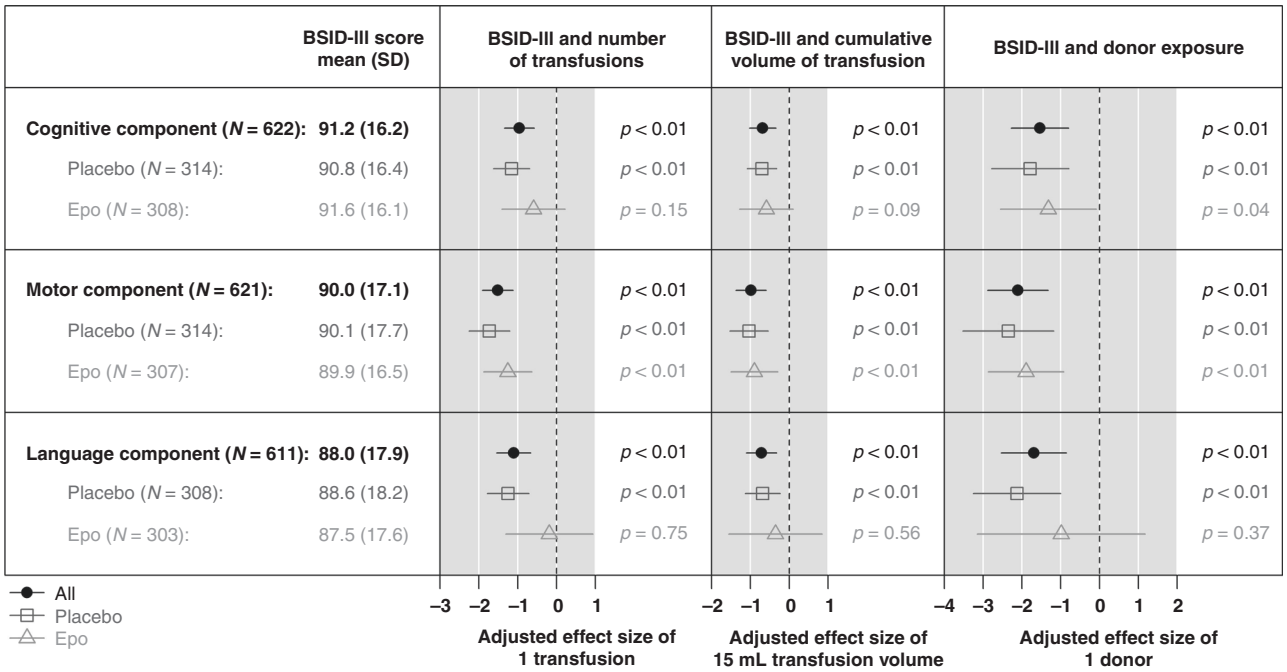


Fig. 2 Primary analysis of BSID-III component scores and pRBC transfusion exposures. The overall associations between mean BSID-III component scores (cognitive, motor, and language) and pRBC transfusion exposures (number of transfusions, cumulative volume of transfusion, and donor exposure) were examined using GEE models clustering on same-birth siblings, adjusted for fixed effects of treatment group, gestational age group, recruitment site, and other potential confounders at baseline. These associations were also evaluated for each treatment group using similar GEE models. The forest plot shows the estimated effect sizes of transfusion exposures on mean BSID-III scores and corresponding 95% CIs.

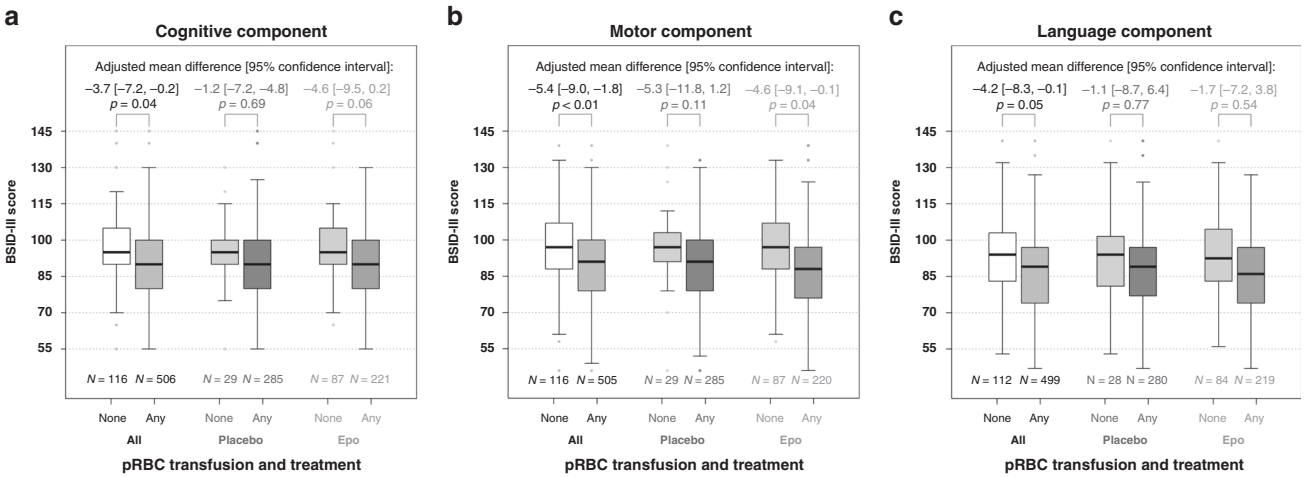
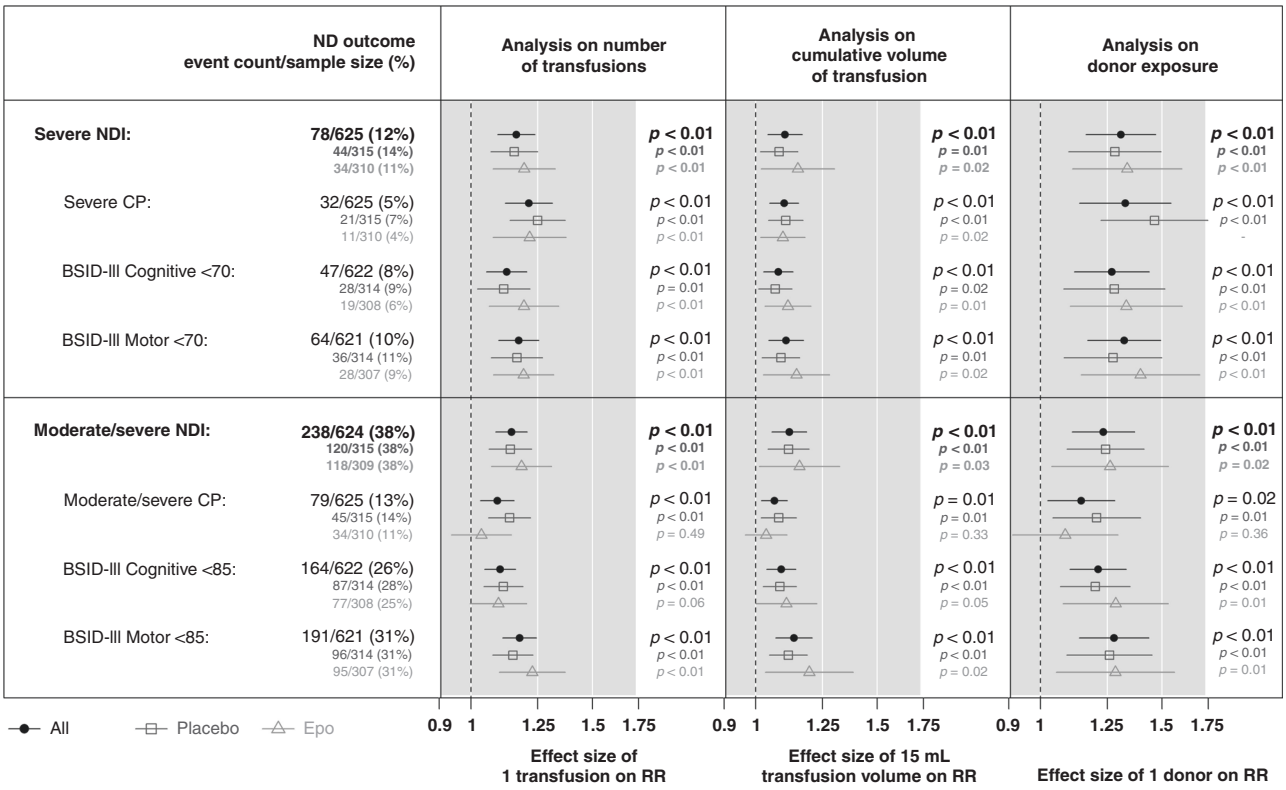


Fig. 3 BSID-III component scores and any pRBC transfusion. The distributions of BSID-III **a** cognitive scores, **b** motor scores, and **c** language scores were summarized for those who were transfusion-free and who had ≥1 pRBC transfusion, overall and by treatment group. The overall associations among all participants were examined using GEE models clustering on same-birth siblings, adjusted for fixed effects of treatment group, gestational age group, recruitment site, and other potential confounding variables at baseline. These associations were also evaluated for each treatment group using similar GEE models.

unique donors. Infants with missing data on either outcomes or any of the adjusted potential confounding covariates (birth weight, Apgar score at 5 min, suspected or confirmed chorioamnionitis, and the use of ventilator for respiratory support at enrollment), as described in Table 1 footnotes, were excluded from statistical analyses.

There was substantial variability in transfusion practice by site. Of the 19 participating sites, 14 (74%) used formal transfusion guidelines. An additional two sites had general guidelines that included suggested volumes for transfusions and standard storage solutions for pRBCs. For critically ill infants requiring mechanical ventilation, hematocrit triggers for transfusion ranged from 28 to 45%. For stable infants, hematocrit triggers ranged from 21 to 25%.

Results of the primary analysis for the associations between transfusion exposures (number of pRBC transfusions, cumulative volume, and donor exposure) and BSID-III scores (cognitive, motor, and language components) are given in Fig. 2. Accounting for potential sibship correlations, treatment effect, site, gestational age, and other potential confounding variables, each additional transfusion received was associated with: a decrease in mean



	All *N*; mean (SD)	Placebo *N*; mean(SD)	Epo *N*; mean(SD)
Number of transfusions	625; 4.3 (4.4)	315; 5.4 (4.8)	310; 3.3 (3.7)
Cumulative volume	625; 65.3 (78.2)	315; 83.7 (87.6)	310; 46.7 (62.0)
Number of unique donors	625; 2.0 (2.1)	315; 2.4 (2.2)	310; 1.6 (1.9)

Fig. 4 Secondary ND outcomes and pRBC transfusion exposures. The overall associations between secondary ND outcomes and pRBC transfusion exposures were examined using GEE loglinear models clustering on same-birth siblings, adjusted for fixed effects of treatment group, gestational age group, and other potential confounding covariates at baseline. These associations were also evaluated for each treatment group using similar GEE models. The forest plot shows the estimated effect sizes of transfusion exposures on the RR of ND outcomes and corresponding 95% CIs. RRs >1.0 indicate that exposure to pRBC transfusions is associated with higher risk of worse ND outcomes.

cognitive score of 0.96 (95% confidence interval (CI) of [−1.34, −0.57], $p < 0.01$); a decrease in mean motor score by 1.51 (95% CI of [−1.91, −1.12], $p < 0.01$); and a decrease in mean language score by 1.10 (95% CI of [−1.54, −0.66], $p < 0.01$). Statistically significant and negative associations were observed for analysis of cumulative volume as well as donor exposure. When stratified by treatment arm, the associations remained statistically significant and negative for all analyses among the placebo infants. For those randomized to Epo, the estimated effect sizes were all negative; however, only the associations between motor score and transfusion exposures were statistically significant.

As a secondary analysis, we examined the associations between BSID-III scores and transfusion status, with the results shown in Fig. 3. Among all infants and in either treatment arm, transfusion-free infants consistently had higher mean scores across all BSID-III components. While the results were statistically significant when considering all subjects, only the motor component for Epo-treated infants was significant when treatment subgroups were examined separately.

We also evaluated the relationships between transfusion exposures and other ND outcomes, which included severe NDI and its components (severe CP, BSID-III cognitive <70, BSID-III motor <70) and moderate-to-severe NDI and its components (moderate-to-severe CP, BSID-III cognitive <85, BSID-III motor <85). The results are presented in Fig. 4. The relative risks (RRs) of an adverse ND outcome were higher among those receiving more transfusions, more cumulative volume, or transfusions from more unique donors. Adjusted for potential confounding covariates, these associations were all statistically significant for all subjects and those in the placebo group. The results were also significant for those in the Epo group, except for moderate-to-severe CP and BSID-III cognitive <85.

Table 2 shows the results for the analysis of adverse ND outcomes and transfusion status. The RRs of an adverse ND outcome were estimated to be higher among those who had any transfusion; however, most of these results did not reach statistical significance. The results of sensitivity analyses using subgroups defined by gestational age or sex are given in Supplemental Table S1 (online). Variability in the associations between BSID-III components and transfusion exposures was seen across different subgroups.

CONCLUSIONS AND DISCUSSIONS

In this post hoc analysis of 628 ELGAN infants from the PENUT Trial who underwent ND assessment at 22–26 months PMA, we found significantly negative associations between BSID-III score and pRBC transfusions, with BSID-III scores progressively decreasing as a function of number of transfusions, cumulative volume of transfusions, and number of donor exposures. These findings were consistent for cognitive, motor, and language components of the BSID III. Similarly, when subjects were

Table 2. Secondary analysis of ND outcomes and any pRBC transfusion.

	All participants ($N = 628$)				Placebo ($N = 315$)				Epo ($N = 313$)			
	No transfusion[a]	Any transfusion[b]	Adjusted RR[c] (95% CI)	p	No transfusion[a]	Any transfusion[b]	Adjusted RR[c] (95% CI)	p	No transfusion[a]	Any transfusion[b]	Adjusted RR[c] (95% CI)	p
Severe NDI	4/116 (3%)	74/512 (14%)	2.9 (1.1, 7.3)	0.03	2/29 (7%)	42/286 (15%)	1.8 (0.5, 6.6)	0.37	2/87 (2%)	32/226 (14%)	3.7 (1.0, 13.3)	0.05
Severe CP[d]	2/116 (2%)	30/512 (6%)	2.4 (0.6, 9.6)	0.20	2/29 (7%)	19/286 (7%)	1.0 (0.3, 3.8)	0.99	0/87 (0%)	11/226 (5%)	—	—
BSID-III cognitive <70	3/116 (3%)	44/509 (9%)	2.1 (0.7, 6.5)	0.18	2/29 (7%)	26/285 (9%)	1.2 (0.3, 4.7)	0.84	1/87 (1%)	18/224 (8%)	3.0 (0.7, 13.6)	0.15
BSID-III motor <70	3/116 (3%)	61/508 (12%)	3.0 (1.0, 9.3)	0.06	1/29 (3%)	35/285 (12%)	2.7 (0.4, 18.5)	0.31	2/87 (2%)	26/223 (12%)	3.0 (0.8, 12.0)	0.11
Moderate/severe NDI	24/116 (21%)	214/511 (42%)	2.1 (1.4, 3.2)	<0.01	6/29 (21%)	114/286 (40%)	2.0 (0.9, 4.1)	0.07	18/87 (21%)	100/225 (44%)	2.2 (1.3, 3.6)	<0.01
Moderate/severe CP	6/116 (5%)	73/512 (14%)	2.4 (1.0, 5.6)	0.05	2/29 (7%)	43/286 (15%)	2.2 (0.6, 8.8)	0.25	4/87 (5%)	30/226 (13%)	1.8 (0.5, 6.0)	0.34
BSID-III cognitive <85	17/116 (15%)	147/509 (29%)	1.9 (1.1, 3.1)	0.01	4/29 (14%)	83/285 (29%)	2.1 (0.8, 5.4)	0.13	13/87 (15%)	64/224 (29%)	1.7 (0.9, 3.2)	0.09
BSID-III motor <85	15/116 (13%)	176/508 (35%)	3.0 (1.8, 5.1)	<0.01	4/29 (14%)	92/285 (32%)	2.3 (0.9, 6.0)	0.08	11/87 (13%)	84/223 (38%)	3.1 (1.5, 6.6)	<0.01

[a]Results were presented as the number of infants (%) with ND outcomes among those without transfusion under each treatment arm.
[b]Results were presented as the number of infants (%) with ND outcomes among those who had ≥1 transfusion under each treatment arm.
[c]The overall relative risk (RR) of secondary ND outcomes (and 95% CI) between two groups defined by transfusion status were evaluated by GEE loglinear models clustering on same-birth siblings, adjusting for treatment, and gestational age at birth. RRs <1.0 favor infants who had no transfusion during follow-up. These associations were evaluated separately for each treatment arm using similar GEE models.
[d]Results for severe CP for the Epo group were not reported, due to unreliable estimate based on zero event in one subgroup.

dichotomized into those who received no pRBC transfusions or those with ≥1 transfusions, lower BSID-III scores were seen in those who had been exposed to pRBC transfusions. Further, the odds of severe or moderate and severe NDI were higher in those with increased transfusion exposure (increased number of transfusions, increased cumulative volume of transfusions, or increased donor exposure) when the cohort was examined as a whole. The association between ND assessment and transfusions was less robust in the Epo-treated arm, with several analyses in the Epo-treated arm not reaching statistical significance. Infants who received transfusions despite being on Epo might have been sicker and therefore unable to respond as robustly to the erythropoietic effect of Epo.

We previously reported cognitive results of preterm survivors enrolled in a randomized controlled trial of ESAs.[9] Cognitive scores at 18–22 months PMA were inversely correlated with transfusion volume; among infants receiving at least one transfusion, cognitive scores were significantly higher in the ESA-treated group ($n = 80$). Although not statistically significant, transfusion number and volume trended toward an inverse correlation to cognitive scores at 3.5–4 years. Scores were higher in the ESA than in the placebo group. Our current, larger study corroborates and expands on these findings.

There remains great longstanding uncertainty regarding appropriate management of anemia in neonates. The potential harm of extreme anemia must be balanced with the potential benefit or harm of transfusions. Severe anemia has been associated with apnea,[20] poor growth,[21] compromised cardiac function,[22] increased mortality,[23] and potentially, poor ND.[9] What constitutes "severe anemia" for the neonatal population, however, is not well defined.[24] In contrast, in the adult literature, the recommended transfusion trigger has fallen to a hematocrit of 21% (hemoglobin of 7) as the adverse effects of transfusion have become more apparent.[25,26] Neonatal transfusions have been associated with many adverse acute effects, including inflammation,[17] extension of intraventricular hemorrhage (IVH),[27] bronchopulmonary dysplasia,[28] retinopathy of prematurity,[29] necrotizing enterocolitis,[29,30] and death.[31] In addition to the short-term effects, the long-term ND effects are also of concern. The randomized controlled trials of liberal vs. stringent transfusion guidelines showed no clear benefit or harm to either approach.[14,20,32] In a post hoc analysis, the Bell study ($N = 100$) showed an increase in severe brain injury (grade-IV IVH or periventricular leukomalacia) in the restrictive group (6 vs. 0). However, up to 2 transfusions were allowed prior to enrollment, the median day of enrollment was 3 days of age, and cranial ultrasounds were not done prior to enrollment, so it is not clear whether the findings reflect the transfusion approach.[32] Follow-up of 44 of the 100 infants enrolled showed cerebral white matter to be substantially reduced in both preterm groups at 12 years of age, but more so for the liberal transfusion group.[12,33] The Premature Infants in Need of Transfusion study ($N = 451$) did not show a difference in acute brain injury, and follow-up of these subjects at 18–20 months corrected age showed no statistical difference in ND outcomes.[10] The ETTNO trial[14] ($N = 1013$) showed no difference in ND outcome measured by BSID-II at 24 months corrected age; however, they did not compare children who received transfusions with those who remained transfusion-free, nor did they evaluate the effect of increasing numbers of transfusions. None of these studies evaluated ESAs in their treatment arms.

The mechanisms by which pRBC transfusions affect development are unclear. Possible explanations include the promotion of the inflammatory cascade,[4,17,34] suppression of endogenous Epo, pro-oxidant injury, and ferroptosis.[35] In this study, the relationship between adverse ND outcomes and transfusion exposure was less robust in the Epo-treated subjects. This may be due to a wider confidence interval in the Epo group, as these subjects received fewer transfusions, which leads to an even smaller number of infants with adverse outcomes and who had transfusions in this

group. Alternatively, as has been shown in preclinical studies,[36] Epo may interrupt or modulate the potential injurious effects of pRBC transfusion.

The primary limitation of this study is that given its post hoc observational nature, it is difficult to ascertain whether increased transfusions are causative of adverse outcomes or rather are merely markers of clinical illness that dictate worse outcomes. We attempted to account for this by adjusting for markers of illness severity, including gestational age, birth weight, 5-min Apgar, chorioamnionitis, and mechanical ventilator use. Additional limitations include the lack of standardized transfusion guidelines (though variation in practice permitted the necessary variability needed to compare the effects of different transfusion exposures) and the absence of data on phlebotomy losses for each infant. Despite these limitations, the PENUT Trial is a well-designed and executed randomized trial with a large cohort of well-characterized participants, which permits exploratory or post hoc analyses such as ours. Our study emphasizes the urgent need to shed further light on this clinically important topic of transfusions and ND, given the frequency at which ELGANs are transfused. This important gap in knowledge will be partially informed by results from the ETTNO and TOP trials, exclusive of the effects of ESAs. The Darbepoetin Trial to Improve Red Cell Mass and Neuroprotection in Preterm Infants (NCT 03169881), will inform this issue, as it does involve an ESA.

We conclude that transfusions in preterm infants may contribute to poor ND outcomes measured at 2 years of age. Strategies to minimize the need for pRBC transfusions, such as delayed cord clamping, judicious phlebotomy practices, appropriate iron supplementation, adoption of transfusion guidelines,[37] and use of ESAs, should be encouraged in order to improve long-term ND in critically ill, ELGANs. These children should be followed for a longer term to determine whether the effects of transfusions observed at 2 years of age persist into school age and beyond.

ACKNOWLEDGEMENTS

This work was supported by NINDS U01 NS077953 and U01 NS077955. NINDS funded the PENUT Trial, but had no role in data collection, analysis, data interpretation, writing of the manuscript, or submission for publication.

FOR THE PENUT CONSORTIUM

PENUT Site PIs: Rajan Wadhawan[4], Sherry E. Courtney[5], Tonya Robinson[6], Kaashif A. Ahmad[7], Ellen Bendel-Stenzel[8], Mariana Baserga[9], Edmund F. LaGamma[10], L. Corbin Downey[11], Raghavendra Rao[12], Nancy Fahim[12], Andrea Lampland[13], Ivan D. Frantz III[14], Janine Khan[15], Michael Weiss[16], Maureen M. Gilmore[17], Nishant Srinivasan[18], Jorge E. Perez[19], Victor McKay[20]

Non-Author Participants:

PENUT Consortium Co-investigators: Billy Thomas[5], Nahed Elhassan[5], Sarah Mulkey[5], Vivek K. Vijayamadhavan[7], Neil Mulrooney[8], Bradley Yoder[9], Jordan S. Kase[10], Jennifer Check[11], Semsa Gogcu[11], Erin Osterholm[12], Sara Ramel[12], Catherine Bendel[12], Cheryl Gale[12], Thomas George[12], Michael Georgieff[12], Tate Gisslen[12], Sixto Guiang III[12], Dana Johnson[12], Katie Pfister[12], Heather Podgorski[12], Kari Roberts[12], Erin Stepka[12], Melissa Engel[12], Heidi Kamrath[12], Johannah Scheurer[12], Angela Hanson[12], Katherine Satrom[12], Elizabeth Zorn[12], Camilia R. Martin[14], Deirdre O'Reilly[14], Nicolas Porta[15], Raye-Ann de Regnier[15], Catalina Bazacliu[16], Frances Northington[17], Raul Chavez Valdez[17], Patel Saurabhkumar[18], Magaly Diaz-Barbosa[19]

PENUT Research Coordinators: Amy Silvia[21], Bailey Clopp[21], John B. Feltner[21], Isabella Esposito[21], Stephanie Hauge[21], Samantha Nikirk[21], Debbie Ott[4], Ariana Franco Mora[4], Pamela Hedrick[4], Vicki Flynn[4], Andrea Purnell[5], Emilie Loy[5], Natalie Sikes[5], Melanie Mason[5], Jana McConnell[5], Tiffany Brown[5], Henry Harrison[5], Denise Pearson[5], Tammy Drake[5], Jocelyn Wright[5], Debra Walden[5], Annette Guy[5], Jennifer Nason[6], Morgan Talbot[6], Kristen Lee[6], Sarah Penny[6], Terri Boles[6], Melanie Drummond[7], Katy Kohlleppel[7], Charmaine Kathen[7], Brian Kaletka[8,13], Shania Gonzales[8,13], Cathy Worwa[8,13], Molly Fisher[8,13], Tyler Richter[8,13], Alexander Ginder[8,13], Brixen Reich[9], Carrie Rau[9], Manndi Loertscher[9], Laura Bledsoe[9], Kandace McGrath[9], Kimberlee Weaver Lewis[9], Jill Burnett[9], Susan Schaefer[9], Karie Bird[9], Clare Giblin[10], Rita Daly[10], Kristi Lanier[11], Kelly Warden[11], Jenna Wassenaar[12], Jensina Ericksen[12], Bridget Davern[12], Mary Pat Osborne[12], Brittany Gregorich[12], Susan Pfister[12], Neha Talele[14], Evelyn Obregon[14], Tiglath Ziyeh[14], Molly Clarke[14], Rachel E. Wegner[14], Palak Patel[14], Molly Schau[15], Annamarie Russow[15], Kelly Curry[16], Susan Sinnamon[16], Lisa Barnhart[16], Charlamaine Parkinson[17], Sandra Beauman[22], Mary Hanson[22], Elizabeth Kuan[22], Conra Backstrom Lacy[22], Edshelee M. Galvis[19], Susana Bombino[19], Arturo Serize[19], Jorge Jordan[19], Denise Martinez[20], Suzi Bell[20], Corrie Long[20]

University of Washington Data Coordinating Center: Mark A. Konodi[21], Christopher Nefcy[21]

PENUT Executive Committee: Roberta Ballard[23], Adam Hartman[24], Scott Janis[24], T. Michael O'Shea[25]

PENUT Medical Monitor: John A. Widness[26]

[4]AdventHealth for Children, Orlando, FL, USA; [5]University of Arkansas for Medical Sciences, Little Rock, AR, USA; [6]University of Louisville, Louisville, KY, USA; [7]Methodist Children's Hospital, San Antonio, TX, USA; [8]Children's Minnesota, Minneapolis, MI, USA; [9]University of Utah, Salt Lake City, UT, USA; [10]Maria Fareri Children's Hospital at Westchester Medical Center, Valhalla, NY, USA; [11]Wake Forest School of Medicine, Winston-Salem, NC, USA; [12]University of Minnesota Masonic Children's Hospital, Minneapolis, MI, USA; [13]Children's Minnesota, St. Paul, MI, USA; [14]Beth Israel Deaconess Medical Center, Boston, MA, USA; [15]Prentice Women's Hospital, Chicago, IL, USA; [16]University of Florida, Gainesville, FL, USA; [17]Johns Hopkins University, Baltimore, MD, USA; [18]Children's Hospital of the University of Illinois, Chicago, IL, USA; [19]South Miami Hospital, South Miami, FL, USA; [20]Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA; [21]University of Washington Seattle, Washington, USA; [22]University of New Mexico, Albuquerque, NM, USA; [23]University of California San Francisco School of Medicine, San Francisco, CA, USA; [24]National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA; [25]University of North Carolina School of Medicine, Chapel Hill, NC, USA and [26]University of Iowa, Iowa City, IA, USA

AUTHOR CONTRIBUTIONS

All authors contributed to all three of the following criteria: (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-01273-w) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Parent consent: Parental consent was obtained prenatally or postnatally, as permitted by each site's IRB.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Fabres, J. et al. Estimating blood needs for very-low-birth-weight infants. *Transfusion* **46**, 1915–1920 (2006).
2. Puia-Dumitrescu, M. et al. Patterns of phlebotomy blood loss and transfusions in extremely low birth weight infants. *J. Perinatol.* **39**, 1670–1675 (2019).
3. Donelan, K. J. & Anderson, K. A. Transfusion-related acute lung injury (TRALI): a case report and literature review. *S. D. Med.* **64**, 85–88 (2011).
4. Dani, C. et al. Red blood cell transfusions can induce proinflammatory cytokines in preterm infants. *Transfusion* **57**, 1304–1310 (2017).
5. Lust, C. et al. Early red cell transfusion is associated with development of severe retinopathy of prematurity. *J. Perinatol.* **39**, 393–400 (2019).
6. Saroha, V., Josephson, C. D. & Patel, R. M. Epidemiology of necrotizing enterocolitis: new considerations regarding the influence of red blood cell transfusions and anemia. *Clin. Perinatol.* **46**, 101–117 (2019).
7. Teiserskas, J., Bartasiene, R. & Tameliene, R. Associations between red blood cell transfusions and necrotizing enterocolitis in very low birth weight infants: ten-year data of a tertiary neonatal unit. *Medicina* **55**, 16 (2019).
8. Gauvin, F. et al. Transfusion-related acute lung injury in the Canadian paediatric population. *Paediatr. Child Health* **17**, 235–239 (2012).
9. Shah, P. et al. Effect of erythropoietin and darbepoetin on transfusions and cognitive development in very low birth weight infants. *E-PAS* **2015**, 1568.503 (2015).

10. Whyte, R. K. et al. Neurodevelopmental outcome of extremely low birth weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. *Pediatrics* **123**, 207–213 (2009).
11. Whyte, R. K. Neurodevelopmental outcome of extremely low-birth-weight infants randomly assigned to restrictive or liberal hemoglobin thresholds for blood transfusion. *Semin. Perinatol.* **36**, 290–293 (2012).
12. Nopoulos, P. C. et al. Long-term outcome of brain structure in premature infants: effects of liberal vs restricted red blood cell transfusions. *Arch. Pediatr. Adolesc. Med.* **165**, 443–450 (2011).
13. Eicher, C. et al. The 'Effects of transfusion thresholds on neurocognitive outcome of extremely low birth-weight infants (ETTNO)' study: background, aims, and study protocol. *Neonatology* **101**, 301–305 (2012).
14. Franz, A. R. et al. Effects of liberal vs restrictive transfusion thresholds on survival and neurocognitive outcomes in extremely low-birth-weight infants: the ETTNO randomized clinical trial. *JAMA* **324**, 560–570 (2020).
15. Juul, S. E. et al. A randomized trial of erythropoietin for neuroprotection in preterm infants. *N. Engl. J. Med.* **382**, 233–243 (2020).
16. Juul, S. E., Mayock, D. E., Comstock, B. A. & Heagerty, P. J. Neuroprotective potential of erythropoietin in neonates; design of a randomized trial. *Maternal Health Neonatol. Perinatol.* **1**, 1–9 (2015).
17. German, K. R. et al. Zinc protoporphyrin-to-heme ratio and ferritin as measures of iron sufficiency in the neonatal intensive care unit. *J. Pediatr.* **194**, 47–53 (2018).
18. Liang, K. Y. & Zeger, S. L. Longitudinal data analysis using generalized linear models. *Biometrika* **73**, 13–22 (1986).
19. R Core Team. *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2018).
20. Kirpalani, H. et al. The Premature Infants in Need of Transfusion (PINT) study: a randomized, controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. *J. Pediatr.* **149**, 301–307 (2006).
21. Keller, A., Hermanussen, M., Vogtmann, C., Kiess, W. & Keller, E. Effect of erythrocyte transfusion on longitudinal bone growth of premature infants assessed by mini-knemometry. *Eur. J. Pediatr.* **158**, 871–872 (1999).
22. Alkalay, A. L., Galvis, S., Ferry, D. A., Simmons, C. F. & Krueger, R. C. Jr. Hemodynamic changes in anemic premature infants: are we allowing the hematocrits to fall too low? *Pediatrics* **112**, 838–845 (2003).
23. dos Santos, A. M. et al. Red blood cell transfusions are independently associated with intra-hospital mortality in very low birth weight preterm infants. *J. Pediatr.* **159**, 371–376 (2011). e371-373.
24. Hardy, J. F. Current status of transfusion triggers for red blood cell concentrates. *Transfus. Apher. Sci.* **31**, 55–66 (2004).
25. Carson, J. L. et al. Transfusion thresholds and other strategies for guiding allogeneic red blood cell transfusion. *Cochrane Database Syst. Rev.* **10**, CD002042 (2016).
26. Carson, J. L. et al. Liberal versus restrictive blood transfusion strategy: 3-year survival and cause of death results from the FOCUS randomised controlled trial. *Lancet* **385**, 1183–1189 (2015).
27. Baer, V. L. et al. Among very-low-birth-weight neonates is red blood cell transfusion an independent risk factor for subsequently developing a severe intraventricular hemorrhage? *Transfusion* **51**, 1170–1178 (2011).
28. Valieva, O. A., Strandjord, T. P., Mayock, D. E. & Juul, S. E. Effects of transfusions in extremely low birth weight infants: a retrospective study. *J. Pediatr.* **155**, 331–337 e331 (2009).
29. Wang, Y. C. et al. Red blood cell transfusion and clinical outcomes in extremely low birth weight preterm infants. *Pediatr. Neonatol.* **58**, 216–222 (2017).
30. Patel, R. M. et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very-low-birth-weight infants. *JAMA* **315**, 889–897 (2016).
31. Ghirardello, S. et al. Effects of red blood cell transfusions on the risk of developing complications or death: an observational study of a cohort of very low birth weight infants. *Am. J. Perinatol.* **34**, 88–95 (2017).
32. Bell, E. F. et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. *Pediatrics* **115**, 1685–1691 (2005).
33. McCoy, T. E. et al. Neurocognitive profiles of preterm infants randomly assigned to lower or higher hematocrit thresholds for transfusion. *Child Neuropsychol.* **17**, 347–367 (2011).
34. Arthur, C. M. et al. Anemia induces gut inflammation and injury in an animal model of preterm infants. *Transfusion* **59**, 1233–1245 (2019).
35. Hirano, K. et al. Blood transfusion increases radical promoting non-transferrin bound iron in preterm infants. *Arch. Dis. Child. Fetal Neonatal. Ed.* **84**, F188–F193 (2001).
36. Wallin, D. J. et al. Neonatal mouse hippocampus: phlebotomy-induced anemia diminishes and treatment with erythropoietin partially rescues mammalian target of rapamycin signaling. *Pediatr. Res.* **82**, 501–508 (2017).
37. Baer, V. L. et al. Implementing a program to improve compliance with neonatal intensive care unit transfusion guidelines was accompanied by a reduction in transfusion rate: a pre-post analysis within a multihospital health care system. *Transfusion* **51**, 264–269 (2011).