Erythropoietin is a glycoprotein originally identified and named for its role in erythropoiesis. Interest in erythropoietin as a neurotherapeutic began in 1993, with preclinical studies showing protective effects of erythropoietin, including decreased apoptosis, inflammation, excitotoxicity, and glutamate toxicity. Erythropoietin was also thought to be important for late brain recovery because it stimulates neurogenesis, angiogenesis, and migration of regenerating neurons. Erythropoietin decreases oxidative stress via upregulation of antioxidant enzymes, inhibition of nitric oxide production, and a decrease in lipid peroxidation. These properties of erythropoietin were thought to be particularly relevant to neuroprotection in newborns, when antioxidant systems are immature. Phase 1 and 2 clinical trials of erythropoietin neuroprotection for both encephalopathy of prematurity and neonatal hypoxic-ischemic encephalopathy demonstrated safety with the promise of efficacy, as demonstrated by meta-analyses of these small studies. Phase 3 randomized clinical trials followed, using a variety of dosing strategies and gestational ages.

Wellmann et al describe the safety and short-term outcomes of the EpoRepair trial, which enrolled 121 preterm neonates born at less than 32 weeks of gestation with diagnosed grade 2 or higher intraventricular hemorrhage between April 1, 2014, and August 3, 2018. Reported interim outcomes include term-equivalent magnetic resonance imaging (MRI) findings and events up to hospital discharge. Infants were randomized by day 8 to a dose of 2000 units/kg of body weight (or placebo) for 5 doses within the first 17 days after enrollment. The rationale for choosing this subset of preterm neonates was that erythropoietin might be more effective in facilitating repair of injury rather than as a prophylactic treatment. No difference was seen in term-equivalent MRI findings or in hospital outcomes, and 5-year outcomes are pending.

Erythropoietin has a long and interesting history with respect to its clinical use. The existence of erythropoietin was first posited in 1906 by Carnot and DeFlandre after the observation that blood transfused from a hemorrhaged rabbit could stimulate red cell production in another rabbit. In 1977, a 30.4-kDa glycoprotein erythropoietic factor was isolated, which allowed for its structural analysis, leading to its cloning in 1985 and production of recombinant erythropoietin, followed quickly by clinical trials for treatment of anemia of chronic kidney disease, and US Food and Drug Administration approval in 1989.

Erythropoietin functions by binding to its homodimeric cell surface receptors, stimulating phosphorylation of Janus kinase 2 and signal transducer and activator of transcription 5 pathways, which are critical in cell survival. Thus, it inhibits apoptosis of immature red blood cells and stimulates their maturation. With respect to its potential use as a neuroprotectant, in 1993, a publication suggested that functional erythropoietin receptors were present on cultured neuronal cell lines. Questions regarding its relevance to the human brain soon ensued. It was determined that erythropoietin was produced by astrocytes in brain, and that the receptor was present on a variety of brain cells including neurons and oligodendrocytes. To explore these potential neurotherapeutic actions, dose finding studies were performed and found that higher erythropoietin doses (500 to 5000 units/kg of body weight per dose) were required for neuroprotection compared with erythropoiesis (200-400 units/kg of body weight per dose) given the requirement to cross the blood brain barrier. However, it should be noted that the vast number of preclinical studies were done in rodents, and there are many important differences between the developing rodent and human brain.

Many trials using erythropoietin in neonatal populations have now been published. The Swiss Neuroprotection Trial randomized 448 neonates born at 26 to 32 weeks of gestation to receive...
3000 units/kg of body weight or placebo for 3 doses given in the first 42 hours after birth. That study showed no benefit on any aspect of neurodevelopment up to age 5 years.8 The Preterm Epo neuroprotection (PENUT) Trial9 enrolled 941 neonates born at 24 to 28 weeks of gestation and used a combination of 5 doses of 1000 units/kg of body weight per dose over the first 2 weeks of life followed by 400 units/kg of body weight per dose 3 times a week through 32 weeks of gestation. That study also demonstrated safety but found no difference in death or neurodevelopmental outcome at 2 years of age. Studies in full-term infants with hypoxic-ischemic encephalopathy have been equally disappointing, as the recently published High Dose Epo for Asphyxia and Encephalopathy (HEAL) Trial (500 neonates) also showed no difference in death or neurodevelopment at 2 years of age.10 Of potential concern, erythropoietin-treated children in the HEAL Trial10 showed a nonspecific increase in serious adverse events and in externalizing behaviors on the Child Behavior Check List. Returning to the EpoRepair trial,2 it is not clear whether adequate iron supplementation was given to prevent iron deficiency, which is common in erythropoietin-treated preterm populations and may affect neurodevelopmental outcomes. Furthermore, the study was not powered to evaluate differences in term equivalent MRI. Although the effects of erythropoietin on long-term neurodevelopment are still pending for this study, the interim results presented by Wellmann et al2 suggest that preterm intraventricular hemorrhage is another condition for which erythropoietin treatment is not an effective neuroprotectant.

What can we learn from these results? First, they highlight the importance of adequately powered phase 3 randomized clinical trials. Meta-analyses of smaller phase 2 trials cannot substitute for well-conducted phase 3 trials and may lead to false conclusions. Second, they highlight the importance of preclinical trials in the development of future neuroprotective agents. Experiments must be adequately powered and must evaluate male and female neonates separately. Furthermore, better preclinical models must be developed that model aspects of the injury, such as chronicity, presensitization with inflammation, and other important clinical factors. These must be done in multiple animal models, including models with gyrified brains, such as ferrets, piglets, sheep, or nonhuman primates. Studies done exclusively in rodents have rarely translated well to humans, with erythropoietin providing a well-described example of the potential failures in translation.

**ARTICLE INFORMATION**

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**Corresponding Author:** Sandra E. Juul, MD, PhD, Division of Neonatology, Pediatrics, University of Washington, 1959 Pacific St, Box 356320, Seattle, WA 98195 (sjuul@uw.edu).

**Author Affiliations:** Division of Neonatology, Pediatrics, University of Washington, Seattle.

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