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NEONATAL THROMBOCYTOPENIA AND MEGAKARYOCYTOPOIESIS

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Abstract

Thrombocytopenia is common among sick neonates, affecting 20–35% of all patients admitted to the neonatal intensive care unit (NICU). While most cases of neonatal thrombocytopenia are mild or moderate and resolve within 7–14 days with appropriate therapy, 2.5–5% of NICU patients develop severe thrombocytopenia, sometimes lasting for several weeks and requiring >20 platelet transfusions. The availability of thrombopoietic agents offers the possibility of decreasing the number of platelet transfusions and potentially improving the outcomes of these infants. Adding thrombopoietin (TPO) mimetics to the therapeutic armamentarium of neonatologists, however, will require careful attention to the substantial developmental differences between neonates and adults in the process of megakaryocytopoiesis and in their responses to TPO. Taken together, the available data suggest that TPO mimetics will stimulate platelet production in neonates, but might do so through different mechanisms and at different doses than those established for adults. In addition, the specific groups of thrombocytopenic neonates most likely to benefit from therapy with TPO mimetics need to be defined, and the potential non-hematological effects of these agents on the developing organism need to be considered. This review summarizes our current understanding of neonatal megakaryocytopoiesis, and examines in detail the developmental factors relevant to the potential use of TPO mimetics in neonates.

I. Neonatal Thrombocytopenia

Platelets first appear in the human fetus at five weeks post-conception, and increase in number during fetal life, reaching a mean of $150 \times 10^9/L$ by the end of the first trimester of pregnancy and normal adult values by 22 weeks of gestation. Since 22 weeks is the lowest gestational age at which a newborn infant is considered viable, this means that even the smallest and most immature infants cared for in neonatal intensive care units usually have platelet counts between 150 and $450 \times 10^9/L$, although platelet counts below $150 \times 10^9/L$ are found in up to 14% of premature infants. Among infants born at term to mothers with normal platelet levels, >98% have platelet counts above $150 \times 10^9/L$. For these reasons, thrombocytopenia in neonates, as in adults, has been traditionally defined as a platelet count $<150 \times 10^9/L$.

This definition was challenged by a recent large population study involving 47,291 neonates treated in a multi-hospital system. In this study, reference ranges for platelet counts at different

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gestational and post-conceptional ages were determined by excluding the top and lower 5th percentile of all platelet counts [1]. Using this approach, the lowest limit (5th percentile) of platelet counts for infants <32 weeks gestation was found to be $104 \times 10^9/L$, compared to $123 \times 10^9/L$ for neonates >32 weeks (Figure 1). While this is the largest study of platelet counts in neonates published to date, it is important to keep in mind that ill neonates were not excluded from this study, so that these values should be regarded as epidemiological “reference ranges” for neonates admitted to the neonatal intensive care unit (NICU), rather than as “normal values” for this population. An additional finding from this study was that the mean platelet counts of the most immature infants (born at 22–25 weeks) always remained below the mean levels measured in more mature infants (Figure 1). The mechanisms underlying these observations are unknown, but they are likely related to developmental differences in megakaryocytopoiesis.

Applying the traditional definition of thrombocytopenia (platelet count $<150 \times 10^9/L$), several studies showed that neonates in general have an incidence of thrombocytopenia at birth slightly below 1% [2]. Among sick neonates, however, the incidence is much higher, ranging from 18 to 35% of infants admitted to the NICU [3,4]. Furthermore, the incidence of thrombocytopenia is inversely proportional to the gestational age of the infants, and reaches approximately 70% among the smallest and most premature infants (those with a birth weight <1000 grams) [5].

In most affected neonates, the thrombocytopenia is mild to moderate, and does not require therapy beyond that of the underlying etiology. However, two recent studies focusing exclusively on severe neonatal thrombocytopenia (defined as a platelet count <50 and $<60 \times 10^9/L$) described an incidence of 2.4 and 5% among all NICU admissions, respectively [6,7]. In both studies, sepsis and necrotizing enterocolitis (NEC) were major causes, and no correlation was found between the severity of the thrombocytopenia and the incidence of *major* bleeding. In the prospective study by Stanworth *et al.*, 15 of 169 (9%) neonates with severe thrombocytopenia had a major hemorrhage. 60% of those hemorrhages were intraventricular (either new IVH or extension of an old IVH), and the rest were pulmonary or gastrointestinal. Nearly 90% of major hemorrhages occurred in premature neonates born at <28 weeks gestation, and 13 of 15 (87%) occurred during the first two weeks of life. Interestingly, the mortality among thrombocytopenic patients correlated with the number of platelet transfusions administered, but not with the severity of the thrombocytopenia.

Neonates with platelet counts $<50 \times 10^9/L$ are usually treated with platelet transfusions in an attempt to diminish the occurrence or the severity of hemorrhages. The rather aggressive approach to thrombocytopenia by neonatologists is explained by the fact that neonates also have the highest incidence of intracranial hemorrhages of any patient group. In fact, 25 to 31% of neonates born with a weight <1500 grams develop an intracranial hemorrhage during the first week of life (the high risk period). However, most of those infants have normal platelet counts at the time of their bleeds [8], thus making the causal correlation between thrombocytopenia and intracranial hemorrhage in very low birth weight neonates difficult to establish. Nevertheless, the importance of thrombocytopenia as a risk factor for major hemorrhage is evidenced by the very high incidence of intracranial hemorrhages (11–20%) among otherwise healthy full-term neonates with neonatal alloimmune thrombocytopenia (NAIT) [9].

Given this background, platelet transfusions are liberally administered in many NICUs. In fact, a recent survey of U.S. and Canadian neonatologists suggested that platelet transfusions are frequently given to sick non-bleeding neonates with platelet counts $>50 \times 10^9/L$ [10]. A number of observational studies also suggest that, in the United States, 9.4% of all NICU patients and more than 50% of extremely-low-birth-weight neonates (<1000g) receive at least one platelet transfusion during their hospital stay [5,11]. More than half of these infants receive two or more platelet transfusions. The most common neonatal conditions that lead to very high use

of platelet transfusions (defined as >20 transfusions) are extracorporeal membrane oxygenation (ECMO) (47%), fungal or bacterial sepsis, NEC (50%), and congenital thrombocytopenias (2%) [12].

Interestingly, no study to date has demonstrated a beneficial effect of platelet transfusions in the neonatal population. On the contrary, several publications have shown a strong association between number of platelet transfusions and high morbidity and mortality among NICU patients with different disease processes [11,13,14]. It is unclear from these studies whether this association simply reflects the fact that sicker patients receive more platelets, or whether platelet transfusions themselves adversely affect outcome, as has been suggested [15]. Nevertheless, the idea to pharmacologically stimulate platelet production in neonates with prolonged and severe thrombocytopenia opens the possibility of decreasing the number of platelet transfusions and potentially improving the outcomes of these infants. Adding TPO mimetics to the therapeutic armamentarium of neonatologists, however, will require careful attention to the developmental differences between neonates and adults in megakaryocytopoiesis and in the response to TPO.

II. Neonatal megakaryocytopoiesis

Over the last decade, a mounting body of evidence has led to the recognition of substantial biological differences between neonatal and adult megakaryocytopoiesis (see Geddis chapter) (Table 1). While TPO appears to be the main thrombopoietic factor throughout life, its biology might be different at various stages of development. Unlike erythropoietin, which changes its main production site from the liver to the kidney during development, TPO is predominantly produced in the liver from early fetal to adult life [16]. For unknown reasons, TPO concentrations are significantly higher in healthy neonates of any gestational age than in healthy adults [17,18].

In regard to megakaryocyte development, several groups have shown that megakaryocyte progenitors of neonates have a higher proliferative potential than those of adults, and give rise to significantly larger megakaryocyte colonies than adult progenitors when cultured *in vitro* [17,19]. Neonatal megakaryocyte progenitors are also present both in the bone marrow (BM) and the peripheral blood (PB), while adult progenitors reside almost exclusively in the BM [20]. Fetal and neonatal megakaryocytes, however, are significantly smaller and of lower ploidy than adult megakaryocytes [21,22], and it has been demonstrated that smaller megakaryocytes produce less platelets than larger megakaryocytes [23]. Based on these observations, it is currently widely accepted that neonates maintain their normal platelet counts on the basis of the increased proliferative rate of their progenitors.

The mechanisms underlying the small size and low ploidy of neonatal megakaryocytes are unclear, but murine transplant studies of neonatal vs. adult progenitor cells into irradiated adult recipient mice have demonstrated that both micro-environmental and cell-intrinsic factors contribute to the neonatal phenotype [24]. Of interest, human subjects transplanted with cord blood stem cells also have significantly smaller BM megakaryocytes (for at least three months following the transplant) than subjects transplanted with adult stem cells, thus confirming the role of cell-intrinsic differences [25]. The molecular mechanisms underlying these differences are only beginning to be elucidated, but recent data suggest that developmental stage-specific microRNA expression profiles might regulate some of these developmental changes [26].

III. The response of neonates to thrombocytopenia

Recognition of these developmental differences in megakaryocytopoiesis led to the question of whether neonates respond to thrombocytopenia in a manner similar to adults, or whether the biological characteristics of their megakaryocytes limit their ability to up-regulate platelet

production during increased demand. In this regard, initial studies observed that preterm neonates with moderate thrombocytopenia due to intrauterine chronic hypoxia exhibited low numbers of marrow megakaryocytes and of circulating megakaryocyte progenitors, yet had significantly lower plasma TPO concentrations than older children or adults with hyporegenerative thrombocytopenias [27,28]. Based on these observations, it was suggested that thrombocytopenic neonates may not increase their plasma TPO levels to the degree that thrombocytopenic adults do. However, a number of subsequent studies reported neonates with thrombocytopenias of different etiologies and serum or plasma TPO concentrations >1,000 pg/mL [29–31]. No studies to date have directly compared circulating TPO concentrations in neonates and adults with thrombocytopenia of similar etiology. Thus, it remains unclear whether neonates truly have a developmental limitation in their ability to upregulate TPO production, or whether the low plasma TPO levels reported in some studies are either due to developmental differences in TPO clearance, or part of the specific pathophysiology of the thrombocytopenia associated with chronic intrauterine hypoxia.

A second important question was whether neonates would increase their megakaryocyte number, size, and ploidy in response to thrombocytopenia. Studies in thrombocytopenic patients and in animal models have shown that, under normal conditions, the *adult* BM responds to increased platelet demand by first increasing the megakaryocyte size and ploidy (within 3–5 days of the stimulus) followed by the megakaryocyte number [32,33]. These changes ultimately lead to a 2 to 8-fold increase in megakaryocyte mass (defined as the product of megakaryocyte concentration and volume). To determine whether thrombocytopenic neonates increase their megakaryocyte mass similarly to adults, a recent study used a combination of immunohistochemistry and image analysis to quantify objectively the megakaryocyte concentration and size in BM samples from thrombocytopenic and non-thrombocytopenic neonates and adults. Findings from this study suggested that thrombocytopenic neonates can increase the number, but not the size, of their megakaryocytes [34], implying a limitation in one of the mechanisms by which platelet production is up-regulated. However, all of the thrombocytopenic adults in the study had chronic immune thrombocytopenic purpura, while the neonates had a variety of etiologies for the thrombocytopenia. Thus, it remained unclear whether the observed differences truly represented developmental limitations, or rather reflected the different underlying disease processes. To address this question, Hu *et al.* recently generated a murine model of fetal immune thrombocytopenia that allowed us to compare the response of fetal and adult mice to consumptive thrombocytopenia. In this study, adult mice treated with an anti-platelet antibody (MWReg30) increased the number and size of their megakaryocytes, primarily in the spleen. Newborn mice exposed in-utero to the same antibody, in contrast, failed to increase their megakaryocyte volume in any of the three hematopoietic organs evaluated [35]. Taken together, these findings support the notion that neonates have a developmental limitation in their ability to increase their megakaryocyte size in response to increased platelet demand.

IV. Effects of TPO on neonatal megakaryocytopoiesis

While considering the introduction of thrombopoietic agents into neonatal medicine, an important question remains whether TPO or TPO mimetics can overcome the developmental limitations described above and increase platelet production in thrombocytopenic neonates. While this remains to be tested, several studies have documented substantial qualitative and quantitative differences in the response of neonatal megakaryocytes and megakaryocyte progenitors to TPO. *In vitro*, TPO stimulates the proliferation of megakaryocyte progenitors at all developmental stages, but neonatal progenitors are significantly more sensitive [17,36]. Specifically, neonatal progenitors generate larger megakaryocyte colonies [19] and reach maximal colony counts at lower TPO concentrations than adult progenitors do (10 vs. 50 ng/mL, respectively), resulting in a significantly larger area under the TPO dose-response curve

[36](Figure 2). In serum-free liquid culture systems, neonatal megakaryocytes also survive and proliferate in response to TPO concentrations as low as 1 ng/mL, which are insufficient to support the survival of adult megakaryocytes [37]. *In vivo*, a single study evaluated the response of non-thrombocytopenic newborn rhesus monkeys to a truncated recombinant form of TPO, known as Pegylated Recombinant Human Megakaryocyte and Development Factor (PEG-rHuMGDF) [38]. In this study, newborn monkeys increased their platelet counts 6- to 12-fold in response to treatment with escalating doses of PEG-rHuMGDF. Although this study did not involve a comparison with adult monkeys, the response of newborn animals to the lowest doses evaluated seemed to be more pronounced than that observed in adult rhesus monkeys in prior studies. Furthermore, the enhanced neonatal response was not explained by pharmacokinetic differences, suggesting that it was likely the result of an increased sensitivity of the neonatal megakaryocyte progenitors.

The effects of TPO on the ploidy of neonatal vs. adult megakaryocytes have also been evaluated. Interestingly, while TPO has been shown to increase the ploidy levels of adult megakaryocytes cultured *in vitro*, it does not support (and might inhibit) the polyploidization of neonatal megakaryocytes (Figure 3) [37]. Despite their low size and ploidy, however, recent data suggest that neonatal megakaryocytes generated in serum-free media supplemented with TPO alone are highly cytoplasmically mature, as demonstrated by the presence of large numbers of alpha-granules and high levels of the maturational marker CD42b [39]. Taken together, the available data suggest that TPO mimetics will potentially stimulate platelet production in neonates, but will do so through different biological mechanisms and at different doses than in adults.

VI. Potential use of TPO mimetics in Neonatology

In 2008, the FDA approved the use of two novel TPO receptor agonists, romiplostim (AMG-531, Nplate®) and Eltrombopag (SB497115, Promacta®), for the treatment of adults with chronic ITP not responsive to standard treatment (see article by D. Kuter in this issue). Although initially restricted to the second-line treatment of ITP in adults patients, it is anticipated that both agents will become part of the treatment for other thrombocytopenic disorders and/or other patient populations. As detailed in the sections above, several factors make thrombocytopenic neonates appealing potential candidates for therapy with these mimetics:

1. They offer the opportunity of decreasing platelet transfusions and potentially improving the outcomes of neonates with severe and prolonged thrombocytopenia;
2. Neonates might have developmental limitations in their ability to increase their megakaryocyte mass, and perhaps their TPO concentrations, in response to increased platelet demand; and
3. Neonatal megakaryocyte progenitors are more sensitive to TPO than adult progenitors, suggesting that doses lower than those used in adults might be sufficient to achieve the desired response.

However, a number of developmental factors will have to be taken into consideration when deciding when and how to introduce these agents into neonatal care. First, it will be critical to determine carefully which thrombocytopenic neonates would benefit from treatment with thrombopoiesis-stimulating agents. In this regard, we know that TPO mimetics, similar to TPO, begin to increase the platelet counts 4–6 days after the start of therapy, reach peak platelet counts at around 10–14 days, and return to baseline by 21–28 days. Thus, only infants whose disease process would predict a platelet transfusion requirement lasting longer than 10–14 days would be appropriate candidates for treatment. Given that approximately 80% of cases of severe thrombocytopenia in the NICU resolve within 14 days [40], this constitutes the minority

of thrombocytopenic neonates. Unfortunately, there are currently no good clinical markers that allow us to predict the duration of thrombocytopenia in affected infants, with the exception of the association between liver disease and prolonged neonatal thrombocytopenia described in two studies [11,41]. Since the liver is the main site of TPO production in neonates and adults, infants with severe liver disease and thrombocytopenia might be attractive candidates. Many infants with congenital viral infections might fall into this category. This approach has already been tested in adults with thrombocytopenia due to HCV-related cirrhosis, in whom treatment with eltrombopag increased the platelet counts and permitted the initiation of antiviral therapy (see chapter by McHutchinson).

A second consideration is the fact that neonates who receive the largest numbers of platelet transfusions (“very high users”, >20 platelet transfusions), most frequently have thrombocytopenia due to ECMO, sepsis, or NEC [12], all conditions associated with high levels of platelet activation. *In vitro* studies have shown that TPO and romiplostim, but not eltrombopag [42], increase the degree of platelet reactivity to agonists. Thus, the theoretical potential of TPO-mimetic agents to increase the risk of thrombosis needs to be carefully evaluated, particularly during the acute phase of clinical conditions already characterized by high levels of platelet activation.

Third, although romiplostim and eltrombopag have exhibited a favorable safety profile in adults, all hematopoietic cytokines have been shown to affect cells and tissues outside of the hematopoietic system, sometimes with consequences related to the specific developmental stage. Erythropoietin, for example, has pro-angiogenic properties on vascular cells that might explain the higher incidence of retinopathy of prematurity found in infants treated with erythropoietin during the first week of life [43]. Similarly, it has been suggested that G-CSF stimulates the proliferation of neuronal stem cells and promotes their differentiation *in vitro*, and Liu et al. recently found the G-CSF-R to be expressed in neuronal stem cells in the murine fetal brain on day E12.5 [44]. The non-hematopoietic effects of TPO are not yet well defined, particularly in a developing organism. However, it is known that TPO and its receptor are expressed in the brain [45], and the available data suggest that TPO might have pro-apoptotic and differentiation-blocking effects on neuronal cells [46,47]. Since the new TPO mimetics, and particularly eltrombopag, have a significantly lower molecular weight than endogenous TPO, they might be more likely to cross the intact blood brain barrier. Thus, a careful evaluation of the potential non-hematopoietic effects of TPO on the developing organism is warranted.

In conclusion, we believe that TPO mimetics bring the promise of reducing platelet transfusions and potentially improving the outcomes of neonates with prolonged and severe thrombocytopenia. However, we also believe that a non-judicious extrapolation of results from adult studies to neonates could represent a significant pitfall. Thus, it is our opinion that these novel thrombopoietic agents should currently be used in neonates only within the context of clinical trials designed to address the challenges posed above, and that take into account the profound differences between neonatal and adult patients.

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References

1. Wiedmeier SE, Henry E, Sola-Visner MC, Christensen RD. Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system. *J Perinatol* 2009;29:130–136. [PubMed: 18818663]

2. Dreyfus M, Kaplan C, Verdy E, Schlegel N, Durand-Zaleski I, Tchernia G. Immune thrombocytopenia working group. Frequency of immune thrombocytopenia in newborns: A prospective study. *Blood* 1997;89:4402–4406. [PubMed: 9192764]
3. Castle V, Andrew M, Kelton J, Giron D, Johnston M, Carter C. Frequency and mechanism of neonatal thrombocytopenia. *J Pediatr* 1986;108:749–755. [PubMed: 3701523]
4. Mehta P, Vasa R, Neumann L, Karparkin M. Thrombocytopenia in the high-risk infant. *J Pediatr* 1980;97:791–794. [PubMed: 7431175]
5. Christensen RD, Henry E, Wiedmeier SE, Stoddard RA, Sola-Visner MC, Lambert DK, et al. Thrombocytopenia among extremely low birth weight neonates: Data from a multihospital healthcare system. *J Perinatol* 2006;26:348–353. [PubMed: 16642027]
6. Baer VL, Lambert DK, Henry E, Christensen RD. Severe thrombocytopenia in the nicu. *Pediatrics* 2009;124:e1095–e1100. [PubMed: 19917581]
7. Stanworth SJ, Clarke P, Watts T, Ballard S, Choo L, Morris T, et al. Prospective, observational study of outcomes in neonates with severe thrombocytopenia. *Pediatrics*. 2009
8. Lupton BA, Hill A, Whitfield MF, Carter CJ, Wadsworth LD, Roland EH. Reduced platelet count as a risk factor for intraventricular hemorrhage. *Am J Dis Child* 1988;142:1222–1224. [PubMed: 3177331]
9. Bussel JB, Sola-Visner M. Current approaches to the evaluation and management of the fetus and neonate with immune thrombocytopenia. *Semin Perinatol* 2009;33:35–42. [PubMed: 19167580]
10. Josephson CD, Su LL, Christensen RD, Hillyer CD, Castillejo MI, Emory MR, et al. Platelet transfusion practices among neonatologists in the united states and canada: Results of a survey. *Pediatrics* 2009;123:278–285. [PubMed: 19117893]
11. Del Vecchio A, Sola MC, Theriaque DW, Hutson AD, Kao KJ, Wright D, et al. Platelet transfusions in the neonatal intensive care unit: Factors predicting which patients will require multiple transfusions. *Transfusion* 2001;41:803–808. [PubMed: 11399824]
12. Dohner ML, Wiedmeier SE, Stoddard RA, Null D Jr, Lambert DK, Burnett J, et al. Very high users of platelet transfusions in the neonatal intensive care unit. *Transfusion*. 2009
13. Kenton AB, Hegemier S, Smith EO, O'Donovan DJ, Brandt ML, Cass DL, et al. Platelet transfusions in infants with necrotizing enterocolitis do not lower mortality but may increase morbidity. *J Perinatol* 2005;25:173–177. [PubMed: 15578029]
14. Bonifacio L, Petrova A, Nanjundaswamy S, Mehta R. Thrombocytopenia related neonatal outcome in preterms. *Indian J Pediatr* 2007;74:269–274. [PubMed: 17401266]
15. Baer VL, Lambert DK, Henry E, Snow GL, Sola-Visner MC, Christensen RD. Do platelet transfusions in the nicu adversely affect survival? Analysis of 1600 thrombocytopenic neonates in a multihospital healthcare system. *J Perinatol* 2007;27:790–796. [PubMed: 17855804]
16. Sola MC, Juul SE, Meng YG, Garg S, Sims P, Calhoun DA, et al. Thrombopoietin (tpo) in the fetus and neonate: Tpo concentrations in preterm and term neonates, and organ distribution of tpo and its receptor (c-mpl) during human fetal development. *Early Hum Dev* 1999;53:239–250. [PubMed: 10088990]
17. Murray NA, Watts TL, Roberts IA. Endogenous thrombopoietin levels and effect of recombinant human thrombopoietin on megakaryocyte precursors in term and preterm babies. *Pediatr Res* 1998;43:148–151. [PubMed: 9432127]
18. Walka MM, Sonntag J, Dudenhausen JW, Obladen M. Thrombopoietin concentration in umbilical cord blood of healthy term newborns is higher than in adult controls. *Biol Neonate* 1999;75:54–58. [PubMed: 9831684]
19. Nishihira H, Toyoda Y, Miyazaki H, Kigasawa H, Ohsaki E. Growth of macroscopic human megakaryocyte colonies from cord blood in culture with recombinant human thrombopoietin (c-mpl ligand) and the effects of gestational age on frequency of colonies. *Br J Haematol* 1996;92:23–28. [PubMed: 8562401]
20. Olson TA, Levine RF, Mazur EM, Wright DG, Salvado AJ. Megakaryocytes and megakaryocyte progenitors in human cord blood. *Am J Pediatr Hematol Oncol* 1992;14:241–247. [PubMed: 1510195]
21. Allen Graeve JL, de Alarcon PA. Megakaryocytopoiesis in the human fetus. *Arch Dis Child* 1989;64:481–484. [PubMed: 2730116]

22. de Alarcon PA, Graeve JL. Analysis of megakaryocyte ploidy in fetal bone marrow biopsies using a new adaptation of the feulgen technique to measure DNA content and estimate megakaryocyte ploidy from biopsy specimens. *Pediatr Res* 1996;39:166–170. [PubMed: 8825404]
23. Mattia G, Vulcano F, Milazzo L, Barca A, Macioce G, Giampaolo A, et al. Different ploidy levels of megakaryocytes generated from peripheral or cord blood cd34+ cells are correlated with different levels of platelet release. *Blood* 2002;99:888–897. [PubMed: 11806991]
24. Slayton WB, Wainman DA, Li XM, Hu Z, Jotwani A, Cogle CR, et al. Developmental differences in megakaryocyte maturation are determined by the microenvironment. *Stem Cells* 2005;23:1400–1408. [PubMed: 16210411]
25. Ignatz M, Sola-Visner M, Rimsza LM, Fuchs D, Shuster JJ, Li XM, et al. Umbilical cord blood produces small megakaryocytes after transplantation. *Biol Blood Marrow Transplant* 2007;13:145–150. [PubMed: 17241920]
26. Gutti RSH, Bailey M, Liu ZJ, Sola-Visner M. Neonatal and adult megakaryocytes have different microRNA expression profiles, which might contribute to their biological differences. *Society of Pediatric Research Meeting E-PAS* 2009;2730:4.
27. Watts TL, Murray NA, Roberts IA. Thrombopoietin has a primary role in the regulation of platelet production in preterm babies. *Pediatr Res* 1999;46:28–32. [PubMed: 10400130]
28. Sola MC, Calhoun DA, Hutson AD, Christensen RD. Plasma thrombopoietin concentrations in thrombocytopenic and non-thrombocytopenic patients in a neonatal intensive care unit. *Br J Haematol* 1999;104:90–92. [PubMed: 10027717]
29. Brown RE, Rimsza LM, Pastos K, Young L, Saxonhouse MA, Bailey M, et al. Effects of sepsis on neonatal thrombopoiesis. *Pediatr Res* 2008;64:399–404. [PubMed: 18552713]
30. Colarizi P, Fiorucci P, Caradonna A, Ficuccilli F, Mancuso M, Papoff P. Circulating thrombopoietin levels in neonates with infection. *Acta Paediatr* 1999;88:332–337. [PubMed: 10229048]
31. Sola MC, Slayton WB, Rimsza LM, Perez JA, Fuch D, Calhoun DA, et al. A neonate with severe thrombocytopenia and radio-ulnar synostosis. *J Perinatol* 2004;24:528–530. [PubMed: 15282617]
32. Harker LA. Kinetics of thrombopoiesis. *J Clin Invest* 1968;47:458–465. [PubMed: 5637135]
33. Harker LA, Finch CA. Thrombokinetis in man. *J Clin Invest* 1969;48:963–974. [PubMed: 5814231]
34. Sola-Visner MC, Christensen RD, Hutson AD, Rimsza LM. Megakaryocyte size and concentration in the bone marrow of thrombocytopenic and nonthrombocytopenic neonates. *Pediatr Res* 2007;61:479–484. [PubMed: 17515875]
35. Hu Z, Slayton WB, Rimsza LM, Bailey M, Sallmon H, Sola-Visner MC. Differences between newborn and adult mice in their response to immune thrombocytopenia. *Neonatology* 98:100–108.
36. Sola MC, Du Y, Hutson AD, Christensen RD. Dose-response relationship of megakaryocyte progenitors from the bone marrow of thrombocytopenic and non-thrombocytopenic neonates to recombinant thrombopoietin. *Br J Haematol* 2000;110:449–453. [PubMed: 10971407]
37. Pastos KM, Slayton WB, Rimsza LM, Young L, Sola-Visner MC. Differential effects of recombinant thrombopoietin and bone marrow stromal-conditioned media on neonatal versus adult megakaryocytes. *Blood* 2006;108:3360–3362. [PubMed: 16888093]
38. Sola MC, Christensen RD, Hutson AD, Tarantal AF. Pharmacokinetics, pharmacodynamics, and safety of administering pegylated recombinant megakaryocyte growth and development factor to newborn rhesus monkeys. *Pediatr Res* 2000;47:208–214. [PubMed: 10674348]
39. Liu ZJBM, Gutti R, Sallmon H, Sola-Visner M. Thrombopoietin stimulates the cytoplasmic maturation of low-ploidy neonatal megakaryocytes through the mtor pathway. *Society of Pediatric Research Meeting E-PAS* 2009;2730.1.
40. Murray NA, Howarth LJ, McCloy MP, Letsky EA, Roberts IA. Platelet transfusion in the management of severe thrombocytopenia in neonatal intensive care unit patients. *Transfus Med* 2002;12:35–41. [PubMed: 11967135]
41. Garcia MG, Duenas E, Sola MC, Hutson AD, Theriaque D, Christensen RD. Epidemiologic and outcome studies of patients who received platelet transfusions in the neonatal intensive care unit. *J Perinatol* 2001;21:415–420. [PubMed: 11894507]
42. Erhardt JA, Erickson-Miller CL, Aivado M, Abboud M, Pillarisetti K, Toomey JR. Comparative analyses of the small molecule thrombopoietin receptor agonist eltrombopag and thrombopoietin on in vitro platelet function. *Exp Hematol* 2009;37:1030–1037. [PubMed: 19631713]

43. Aher SM, Ohlsson A. Early versus late erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane database of systematic reviews (Online) 2006;3 CD004865.
44. Liu H, Jia D, Fu J, Zhao S, He G, Ling EA, et al. Effects of granulocyte colony-stimulating factor on the proliferation and cell-fate specification of neural stem cells. *Neuroscience* 2009;164:1521–1530. [PubMed: 19782730]
45. Dame C, Wolber EM, Freitag P, Hofmann D, Bartmann P, Fandrey J. Thrombopoietin gene expression in the developing human central nervous system. *Brain Res Dev Brain Res* 2003;143:217–223.
46. Ehrenreich H, Hasselblatt M, Knerlich F, von Ahsen N, Jacob S, Sperling S, et al. A hematopoietic growth factor, thrombopoietin, has a proapoptotic role in the brain. *Proc Natl Acad Sci U S A* 2005;102:862–867. [PubMed: 15642952]
47. Samoylenko A, Byts N, Rajalingam K, von Ahsen N, Rapp UR, Ehrenreich H, et al. Thrombopoietin inhibits nerve growth factor-induced neuronal differentiation and erk signalling. *Cellular signalling* 2008;20:154–162. [PubMed: 18006272]

Figure 1. Effect of postnatal age on the mean platelet counts of premature infants

Mean platelet counts were plotted according to gestational age at birth, and data were grouped according to weeks of gestation completed at birth, as follows: 22–24 weeks, 25 to 26 weeks, 27 to 28 weeks, 29 to 30 weeks, 31 to 32 weeks, and 33 to 34 weeks. As can be seen, mean platelet counts increased with post-natal age in all groups, but the most premature infants (22–24 weeks, blue line) had mean platelet counts persistently lower than those of the more mature infants. **Reprinted from:** Christensen RD, et al. The CBC: reference ranges for neonates. *Semin Perinatol* 2009;33:3–11, with permission.

Figure 2. In vitro relationship of maximal megakaryocyte colony count and recombinant (r) human TPO concentration

Bone marrow from thrombocytopenic (T) and non-thrombocytopenic (NT) neonates and adults was cultured in the presence of increasing concentrations of rTPO and the number of megakaryocyte colonies counted. Neonatal progenitors reached a plateau at 10 ng/ml and had a larger area under the curve than did adult progenitors, which reached a plateau at 50 ng/ml.

Reprinted from: Sola MC, et al. Dose-response relationship of megakaryocyte progenitors from the bone marrow of thrombocytopenic and non-thrombocytopenic neonates to recombinant thrombopoietin. *Br J Haematol* 2000; 110:449-53, with permission.

Figure 3. The percentage of megakaryocytes $\geq 8N$ in peripheral blood (PB) and cord blood (CB) cultures differed depending on media source and rTPO concentration

PB- and CB-CD34⁺ cells were cultured for 14 days in serum-free unconditioned medium (UCM) and bone marrow stromal conditioned medium (CM), with varying rTPO concentrations. PB-derived megakaryocytes (solid lines) cultured in UCM (A) exhibited a rTPO dose-dependent increase in ploidy levels, an effect not observed in the presence of CM (B). CB-derived megakaryocytes (dashed lines) reached highest ploidy levels when cultured in CM with no rTPO, and effect that was reversed by increasing rTPO concentrations ≥ 1 ng/ml (B). Data shown represent the means and standard error of the mean (SEM) of four separate experiments. **Reprinted from:** Pastos K, et al. Differential effects of recombinant thrombopoietin and bone marrow stromal-conditioned media on neonatal versus adult megakaryocytes. *Blood* 2006; 108: 3360-2, with permission.

Table 1

Major differences in megakaryocytopoiesis between neonates and adults.

	Neonates	Adults
TPO concentrations	Slightly higher in healthy neonates than in healthy adults	Lower in healthy adults than in healthy neonates
MK progenitors	Abundant in the blood Give rise to large colonies More sensitive to TPO	Sparse in the blood Give rise to small colonies Less sensitive to TPO
MKs	Small Low ploidy levels	Large High ploidy levels
Effects of rTPO	Inhibits MK polyploidization	Stimulates MK polyploidization

Adapted from: Sola-Visner MC. Thrombocytopenia in the NICU: New Insights into causative mechanisms and treatments. *Haematologica reports* 2006;2:65–69. MK: megakaryocyte.