Granulocyte-colony stimulating factor for hematopoietic stem cell donation from healthy female donors during pregnancy and lactation: what do we know?

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Submitted on August 3, 2012; resubmitted on October 14, 2012; accepted on November 27, 2012

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BACKGROUND: Hematopoietic growth factors (HGFs) are mostly used as supportive measures to reduce infectious complications associated with neutropenia. Over the past decade, the use of HGFs became a common method for mobilizing human CD34+ stem cells, either for autologous or allogeneic transplantation. However, since their introduction the long-term safety of the procedure has become a major focus of discussion and research. Most information refers to healthy normal donors and data concerning pregnant and lactating women are scarce. The clinical question, which is the core of this review, is whether stem cell donation, preceded by administration of granulocyte-colony stimulating factor (G-CSF) for mobilization, is a safe procedure for pregnant donors.

METHODS: Literature searches were performed in Pubmed for English language articles published before the end of May 2012, focusing on G-CSF administration during pregnancy, lactation and hematopoietic stem cell donation. Searches included animal and human studies.

RESULTS: Data from animals (n = 15 studies) and women (n = 46 studies) indicate that G-CSF crosses the placenta, stimulates fetal granulopoiesis, improves neonatal survival mostly for very immature infants, promotes trophoblast growth and placental metabolism and has an anti-abortive role. Granulocyte macrophage-CSF is a key cytokine in the maternal immune tolerance towards the implanted embryo and exerts protective long-term programming effects to preimplantation embryos. The available data suggest that probably CSFs should not...

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be administered during the time of most active organogenesis (first trimester), except perhaps for the first week during which implantation takes place. Provided CSF is administered during the second and third trimesters, it appears to be safe, and pregnant women receiving the CSF treatment can become hematopoietic stem cell donors. There are also risks related to the anesthesia, which is required for the bone marrow aspiration. During lactation, there should be a period of at least 3 days to allow for clearance of CSF from milk before resuming breast feeding. With regard to teratogenicity or leukaeemogeneity, in non-pregnant or non-lactating women reports show that CSF administration is associated with a risk for leukemia; however, this risk is not higher compared with the control population.

**Conclusions:** The information available to date indicates that administration of CSF in general, and G-CSF in particular, is safe and healthy pregnant women can serve as donors of either bone marrow or peripheral blood stem cells. However, the clinical experience is rather limited and therefore until more data become available, G-CSF should not be used during pregnancy and lactation when other therapeutic options, instead of stem cell transplantation, are available.

**Key words:** granulocyte-colony stimulating factor / pregnancy / lactation / stem cell transplantation

## Introduction

Granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) are proteins known as hematopoietic growth factors (HGFs) or colony-stimulating factors (CSFs). They are secreted glycoproteins that bind to receptor proteins on the surface of hemopoietic stem cells, thereby activating intracellular signaling pathways and regulating the proliferation and differentiation of hematopoietic progenitor cells of the neutrophilic series, as well as enhancing the function of mature neutrophils (Cohen et al., 1987; Ulich et al., 1988). HGFs are mostly used in clinical practice as supportive measures to reduce infectious complications associated with congenital or acquired neutropenia.

The most commonly clinically used CSF is filgrastim. Filgrastim is a water-soluble 173 amino acid protein with a molecular weight of ~19 kD. It is obtained from the bacterial fermentation of a strain of Escherichia coli transformed with a genetically engineered plasmid containing the human G-CSF gene. It has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for the expression in E. coli (Zsebo et al., 1986). There is a positive linear correlation between the dose and the serum concentration of filgrastim, whether administered intravenously or subcutaneously (Kotto-Kome et al., 2004).

Over the past decade, the use of CSFs and especially G-CSF became a common and reproducible way for mobilizing human CD34+ stem cells, either for autologous or allogeneic transplantation. However, since their introduction as a way of mobilizing progenitor cells, the long-term safety of the procedure has become a major focus of discussion and research. Most information refers to healthy donors and data concerning pregnant and lactating women are scarce. Although current information is insufficient to establish guidelines and recommendations, considerations related to the administration of CSFs and G-CSF during pregnancy and lactation include an unknown risk of spontaneous abortion, and embryonic and fetal malformations, as well as concerns about potential long-term effects, such as teratogenicity or leukaeemogeneity.

The current review aims to provide a better understanding of the biology and the potential direct and indirect effects of the administration of G-CSF growth factor during pregnancy and lactation, in order to initiate consensus and formulation of guidelines and recommendations. The review deals with the most relevant clinical aspects of G-CSF use, bringing together findings about how this growth factor administered in selective instances during pregnancy can have beneficial effects and, more importantly, the specific clinical indications for doing so. Furthermore, the conclusions and recommendations of the review are based on an extensive literature search of various types of journals, from hematology to pediatrics and to obstetrics and gynecology, and therefore are applicable to a broad array of practitioners and researchers who we believe would benefit from a summary of the information on this specific topic.

The first part of the review presents the data related to the physiological background of G-CSF administration and pregnancy, and discusses pregnancy outcomes as well as the challenging issues of miscarriage risk and modulation of implantation competence and post-implantation development (gestational regulation). The second part of the review focuses on the G-CSF administration for hematopoietic stem cell donation during pregnancy and lactation, summarizing and discussing available data on peripheral blood stem cell (PBSC) and bone marrow normal pregnant donors (few reports), lactation, long-term effects and leukaeemogeneity risk.

## Methods

We compiled relevant English language articles that were published before the end of May 2012 and were accessed through a Pubmed database search. The selection criteria aimed at covering a wide range of issues related to G-CSF administration in pregnancy.

For the first part of the review on the physiological background of G-CSF administration and pregnancy, combinations of the following search terms were used: HGFs, CSFs, G-CSF, GM-CSF, pregnancy, neutropenia, granulopoiesis, miscarriage, gestation. For the second part, on G-CSF administration and hematopoietic stem cell donation during pregnancy and lactation, combinations of the following search terms were used: HGFs, CSFs, G-CSF, GM-CSF, stem cell donation, peripheral blood stem cell mobilization, bone marrow harvesting, pregnancy, lactation, teratogenicity, leukaeemogeneity. In addition, references cited in the retrieved articles were also searched.

Initially, more than 500 articles were retrieved from Pubmed. Studies focusing on possible short-term side effects or any other actions of CSF administration that were not relevant to pregnancy, lactation or hematopoietic stem cell donation were excluded (450 articles). Eventually 63 articles (RCTs, cohort studies and case reports) cited in journals with a good impact factor, independent of the number of subjects involved, with either animal (15 articles) or human (46 articles) subjects, and on the basis of
G-CSF or GM-CSF administration for any reason during pregnancy, lactation and/or bone marrow or PBSC donation, were eligible for inclusion in this review. A further two articles provided data on GCSF pharmacokinetics.

The review was designed to bring together a body of literature on GCSF and pregnancy that has been scattered throughout many different types of journal and therefore perhaps not reached all practitioners and researchers.

**Physiological background of G-CSF administration and pregnancy**

**G-CSF in rat models of pregnancy**

We first addressed the available animal models. In 1993, Medolock et al., showed in two cohort studies that G-CSF can cross the placenta of rats, stimulate fetal granulopoiesis and improve neonatal survival after a group B streptococcal infection (Medolock et al., 1993; Novales et al., 1993). In more detail, maternal and fetal serum G-CSF levels were determined before and after G-CSF administration to the mother. Native serum G-CSF levels were undetectable. After a single pulse of G-CSF administration to 20-day gestation rats, rapid appearance of G-CSF was detected both in the serum of the mother and the full-term fetuses (Medolock et al., 1993; Novales et al., 1993). Daily treatment with extremely low concentrations of G-CSF followed, up to 6 days before parturition (15–21 gestation days). As a result the myelopoietic status of the fetuses was affected by induction of neutrophilia in the blood and an increase in the storage pool of neutrophils in the marrow, suggesting possible enhancement of the defense mechanism of the neonates against bacterial infections (Medolock et al., 1993). In a complementary report the same team demonstrated that neonatal rats which had been treated with G-CSF in utero (6 days before parturition) were indeed protected against an otherwise lethal challenge of group B streptococcal infection (Novales et al., 1993).

**G-CSF administration during pregnancy in order to improve congenital and/or cyclic neutropenia**

G-CSF and GM-CSF enhance effectively both the number and the function of mature neutrophils. Therefore, a logical clinical application of G-CSF in pregnant women and an attempt to improve congenital and/or cyclic neutropenia. Indeed, several reports were published on this subject. Calhoun and Christensen administered during a cohort study a single IV dose of G-CSF (25 μg/kg) to 26 women before pre-term delivery (< or = 30 weeks gestation). They reported that G-CSF maternal administration could increase fetal neutrophil production and improve neonatal outcome without significant immediate adverse effects on mother or infant (Calhoun and Christensen, 1998). Kaufmann et al. reported the first successful G-CSF daily administration to a term delivery woman with severe congenital neutropenia. G-CSF was injected from Week 37 of gestation. The pregnancy progressed uneventfully and at Week 38 a healthy boy with a normal cell count was delivered (Kaufmann et al., 1998). In another case report, Abe et al. administered G-CSF at 39 weeks of pregnancy to a 24-year-old pregnant woman with cyclic neutropenia who also delivered a healthy baby without complications (Abe et al., 2000).

Similarly, Sangalli et al. administered G-CSF at 37 weeks of gestation to a 30-year-old pregnant woman with chronic severe acquired neutropenia. A healthy boy who was breastfeeding was discharged after 4 days and the birth was an uncomplicated vaginal term delivery without puerperal complications or side effects (Sangalli et al., 2001).

**HGF administration during pregnancy in order to stimulate neonatal granulopoiesis**

The next clinically important indication is stimulating neonatal granulopoiesis. Not surprisingly, G-CSF administration was reported by Ahmad et al. to improve neutrophil count in critically ill premature neonates, faster than their endogenous cytokine production, and much faster than GM-CSF administration (Ahmad et al., 2002). Out of the 28 patients, 10 received G-CSF (5 μg/kg/dose iv twice a day), 10 received GM-CSF (4 μg/kg/dose iv twice a day) and 8 received placebo, all for a maximum of 7 days or until an absolute neutrophil count (ANC) of 10 000 cells/mm³ was reached. A significant increase in the ANC above the baseline was present on Day 2 in the G-CSF group and on Day 5 in the GM-CSF and placebo groups. No signs of acute toxicity related to the growth factor administration were reported; however, mortality and morbidity were unchanged (Ahmad et al., 2002).

G-CSF increased neonatal neutrophil survival by delaying apoptosis but did not significantly alter neutrophil function. On the other hand, GM-CSF did not significantly delay apoptosis but enhanced this by up-regulating both CD11b expression and the activity of the reactive oxygen intermediates, according to Molloy et al. (2005).

An additional potential indication is prophylactic treatment of neutropenic premature neonates with G-CSF, for incidence of nosocomial infections. Kuhn et al. on a multicenter, randomized, placebo-controlled trial reported that the administration of G-CSF (10 μg/kg/day) for 3 days to premature neonates had a significant prophylactic effect during the first 2 weeks of post-treatment but had no significant effect after 4 weeks (Kuhn et al., 2009). It was also reported that less mature infants benefit from the treatment more than the older ones, thus confirming the conclusions of Funke et al. who emphasized that G-CSF could help the youngest and the smallest infants (Funke et al., 2000).

**G-CSF and miscarriage**

As shown above, G-CSF is indicated and effective in several clinical indications, including during pregnancy. However, what about safety? Safety concerns were always raised and first of all the risk of miscarriage. On the other hand, a positive effect of G-CSF on trophoblast growth and placenta metabolism has been reported (McCracken et al., 1996, 1999). The regulated development of the placenta is critical for fetal growth and maturation, and normal placental development relies upon the controlled invasion of fetal trophoblast into the spiral arteries in the maternal decidual tissue during the early stages of placenta (Lewis et al., 2012). In early reports, the expression of G-CSF has been found on trophoblast and also in decidual cells of placenta in several mammals, including human. The G-CSF receptor was indeed localized only on the trophoblast cell surface (McCracken et al., 1996). In animals, an anti-abortive role was found for G-CSF (Litwin et al., 2005). In accordance, trophoblast of human early miscarriage failed to express G-CSF (Litwin et al., 2005).
Of notice were reports in experimental animals indicating G-CSF as causing placental embolism and consequent abortions caused by peripheral leukocytosis (Keller and Smalling, 1993). This was noticed, however, only in rabbits who received a very high dose (200 μg/kg/day) of G-CSF (Kato et al., 2001), whereas in rats, mice and monkeys no adverse effects were observed (Okasaki et al., 2002). Reports on humans (Table I) from the Severe Chronic Neutropenia International Registry (SCNIR), one in 2002 (Cottle et al., 2002) and the other in 2003 (Dale et al., 2003), discuss the outcomes of 23 pregnancies of women who were treated with G-CSF. During clinical trials, three women became pregnant and although they were excluded from the study, continued to receive commercially available G-CSF (Cottle et al., 2002). Of these three women, two with cyclic neutropenia had normal infants and the third with idiopathic neutropenia had an elective abortion because of abnormal bleeding but she subsequently died of a thrombotic event, probably related to the pregnancy itself and not G-CSF administration (Cottle et al., 2002). The SCNIR also collected data on 20 pregnancies of women who had been exposed to G-CSF and 105 pregnancies of women who were not exposed to the agent (historical controls) (Cottle et al., 2002; Dale et al., 2003). The outcomes of the G-CSF-exposed group, treated for an average of two trimesters (range one to three) at an average dose of 2.7 μg/kg/day (range: 0.2–12), were 13 normal infants, 3 spontaneous abortions and 4 elective abortions. Among the 105 historical controls, there were 77 live births, 24 spontaneous abortions and 8 elective abortions, which did not differ significantly from the exposed group (Cottle et al., 2002; Dale et al., 2003). Thus, the overall rate of pregnancy termination among G-CSF-treated women versus non-treated women was similar.

As CSFs and G-CSF are important for placental growth and development, their therapeutic role against miscarriage was recently evaluated Scarpellini et al. examined the therapeutic benefit of G-CSF administration to women with unexplained primary recurrent miscarriage (Table I). Out of 68 selected women with unexplained primary recurrent miscarriage, 35 received G-CSF starting on the sixth day after ovulation, while 33 women did not receive. In the group treated with G-CSF, 29 out of 35 women delivered a healthy baby (82.8%), whereas in the placebo group only 16 out of 33 did (48.5%) (P: 0.0061 by two-tailed Student’s t-test, Fisher’s exact test and χ²), indicating that G-CSF is a promising treatment in women with unexplained recurrent miscarriage (Scarpellini and Sbracia, 2009). Also, significantly higher β-HCG levels were found in the 5th–9th weeks of gestation in women treated with G-CSF versus placebo (P < 0.001 by two-tailed Student’s t-test, Fisher’s exact test and χ²) suggesting a direct trophic effect of G-CSF on the trophoblast cell, probably mediated by its natural receptor c-fms expressed in the trophoblast (Uzumaki et al., 1989). Once more, no major side effects were observed except for a mild local skin rash which cleared in a few days and two cases of leukocyte count higher than 25 000/ml.

### GM-CSF and gestational regulation

Another important and challenging issue is gestational regulation. As the preimplantation embryo traverses the female reproductive tract and develops from the zygote to blastocyst stage, it experiences fluctuations in the physiochemical composition of its extracellular environment, including the availability of nutrients, growth factors and cytokines. Growth factors and cytokines, such as GM-CSF, mediate signalling between the maternal tissues and the embryo and act in the embryo to modulate implantation competence and post–implantation development (O’Neill, 2008).

GM-CSF is secreted by epithelial cells lining the oviduct and the uterus (Robertson et al., 1992) and is a key cytokine in the uterus, influencing the immune response to pregnancy. That is so because GM-CSF expression is strongly induced during the controlled inflammatory response elicited by male seminal fluid at copitus (Robertson et al., 1996). As a result, activation of T cells, reactive with paternal antigens, occurs within 72 h (Moldenhauer et al., 2009) and is associated with the expansion of the T regulatory (Treg) cell pool that confers immune tolerance to the implanted embryo 4 days following implantation (Robertson et al., 2009). Disruption of the immune adaptation initiated during this period causes failure of ‘allogeneic’ pregnancy (Aluvihare et al., 2004).

In vitro studies also indicate that GM-CSF is a potent embryotrophic factor with survival- and development-promoting effects on both mouse and human embryos (Sjoblom et al., 1999; Robertson et al., 2001). The most obvious effect of GM-CSF on human and mouse blastocysts in vitro is the induction of an increased number of blastomeres, as well as the increased glucose uptake (Robertson et al., 2001).

Finally, embryo-transfer experiments in mice show that GM-CSF exerts long-term programming effects on preimplantation embryos. The addition of GM-CSF to the culture medium protected mice embryos from later adverse effects of in vitro culture, including restriction of fetal growth and incidence of metabolic disorders in adult progeny (Sjoblom et al., 2005).
G-CSF administration and hematopoietic stem cell donation during pregnancy and lactation

Reports of G-CSF administration to healthy pregnant PBSC donors

Following the presentation of the physiological background and the extensive animal work we are now ready to pose the clinical question which is the core of this review. Namely, is stem cell donation, preceded by administration of G-CSF for mobilization, a safe procedure for pregnant donors? Reports on administration of G-CSF to PBSC normal pregnant donors are sparse.

One report is on a 29-year-old woman who underwent a 4-day course of G-CSF subcutaneous administration (10 μg/kg/day) in order to donate PBSCs to her 23-year old brother suffering from non-Hodgkin lymphoma. At a control examination, 3 months after PBSC donation, it was found that she had been pregnant without knowing at the time of mobilization. The gestation age was calculated to be 8 weeks when the G-CSF administration was started. The pregnancy progressed uneventfully and after 40 weeks of gestation, a healthy male baby was delivered at term. Repeat pediatric examinations until the age of 18 months showed no evidence of haematological alterations (Leitner et al., 2001).

In another case report, a 37-year-old woman at 21 weeks gestation was diagnosed with acute myeloid leukemia. She decided to maintain the pregnancy and underwent induction therapy. On Day 14 of the consolidation therapy, while in the 29 week of gestation, she received a 6-day course of G-CSF subcutaneous administration (16 μg/kg/day) to enhance stem cell mobilization. At 37 weeks of gestation a viable female infant was delivered (Niedermeier et al., 2005).

During which trimester should administration of G-CSF be safest? During the first trimester, is when most of the internal and external structures of the fetus are formed and the cells are growing quickly (organogenesis period). Therefore, administration of drugs at that period carries a higher risk of fetal malformation and fetal loss (Brewer et al., 2011; Pollheimer and Knöfler, 2012). With a few exceptions (such as the brain and the reproductive system) most of the fetal organ system development is complete by the beginning of the second trimester. However, exposure to several chemotherapeutic drugs in the second and third trimesters has been associated with a greater risk for premature birth, low birthweight and a temporary reduction in some of the baby’s blood cells (Brewer et al., 2011). In view of the above information and because of the lack of extensive data, it seems safer to avoid G-CSF during first trimester.

Bone Marrow harvesting from pregnant normal stem cell donors

What about bone marrow harvesting? Calder et al. reported three cases of normal donors who underwent successful bone marrow harvest while pregnant. The first was a 21 years old in the 8th week of gestation, the second was 20 years old in the 28th week of gestation and the third was 24 years old, also in 28th week of gestation: the amount of bone marrow harvested was 125, 260 and 350 ml, respectively. The harvesting was performed via multiple bilateral iliac crest aspirations, under epidural or spinal anesthesia and in prone or left lateral decubitus position. In all these cases, pregnancy progressed uneventfully and all donors delivered healthy babies at term (Calder et al., 2005). What stands out from these three rare cases is the fact that in bone marrow harvesting during pregnancy: (i) general anaesthesia should be avoided given the increased risk for pulmonary aspiration of gastric contents, (ii) risk of fetal distress should be decreased via adequate intravenous fluid administration and by minimizing harvest volume, (iii) harvest should be delayed until ~28 weeks of gestation to improve the likelihood of neonatal survival in the event of premature delivery. Provided all the above are accomplished, a successful bone marrow harvest during pregnancy is possible (Calder et al., 2005).

G-CSF and lactation

The next relevant question is what about G-CSF and lactation? This is particularly important as breast feeding has become more popular in recent years owing to its vast importance to the health and development of the newborn, boosting the developing immune system and the defences for combating infection episodes. When a nursing woman is treated with G-CSF it could be excreted in her milk and thus affects her infant. To our knowledge, there are only two reports on G-CSF kinetics in the milk of nursing women who received G-CSF treatment for harvesting PBSCs (Shibata et al., 2003; Kaida et al., 2007). In the first report, the G-CSF level in the human milk was measured at three time points (on Days 4, 5 and 6) in a donor receiving a 5-day administration of G-CSF. The authors reported that G-CSF was still detectable even 24 h after the end of the administration (Shibata et al., 2003). The second report went a step further. G-CSF was administered subcutaneously to a 25-year-old nursing donor for 6 days and G-CSF levels in milk were measured frequently. The level increased gradually, then decreased slowly after 43 h and became undetectable 70 h after the end of the G-CSF administration (Leitner et al., 2001). As the effects of G-CSF in human milk on neonates are not known, the offering of human milk during maternal receipt of G-CSF was proposed to be deferred until at least 3 days after the end of the G-CSF treatment (Kaida et al., 2007).

As data on the issue of oral bioavailability of G-CSF from mother to lactating neonate are scarce, some additional information can be found from pegylated bovine G-CSF administration in the bovine model from the European Public Maximum Residue Limit assessment report for reducing the incidence of clinical mastitis in periparturient cows [European Public MRL assessment report (EPMAR), 2012]. As a result bovine G-CSF is a constituent of the normal human diet of those who eat meat.

However, substances like bovine G-CSF can be expected, even when pegylated, not to be orally bioavailable and to be degraded into their constituent peptides/amino acids through the normal process of digestion, in which case no consumer safety concerns would arise. Therefore, the risk assessment of pegylated bovine granulocyte (PEGbG)-CSF was primarily based on data gained from an oral bioavailability study in rats. Groups of three male and three female rats received PEGbG-CSF as a single dose, either subcutaneously or orally. A control group treated subcutaneously with formulation buffer was included. For all groups, blood samples were collected prior to...
dosing and at 0.25, 1, 2, 4, 12, 24, 48, 72, 96 and 120 h after administration. The quantitative analysis via electrochemiluminescent immunoassay showed that PEGbG-CSF levels at the pre-dose time point were consistently below the lower limit of quantification, or below 46.9 ng/ml, for all animals regardless of the treatment group. Over the 120-h course of the study, it became apparent that rats accumulated up to ~20,000 ng/ml PEGbG-CSF in serum following subcutaneous treatment, whereas rats treated with control article and orally with PEGbG-CSF showed no measurable levels of PEGbG-CSF in serum. A comparison of the subcutaneous AUC (area under the curve) of ~42,000 ng.hour/ml (calculated for 0–72 h) and the worst-case scenario for the AUC for the oral dose (i.e. assuming that all values are at the limit of quantification of 46.9 ng/ml) indicated that the relative oral bioavailability was 0.08%. From this it was concluded that the oral bioavailability of PEGbG-CSF is negligible [European public MRL assessment report (EPMAR), 2012].

### G-CSF administration in pregnancy and long-term effects

Until recently, pregnancy was considered a relative contraindication for G-CSF-induced PBSC donation. Considerations against the administration of G-CSF include an unknown risk of spontaneous abortion, and of embryonic or fetal malformations, as well as concerns about potential long-term effects, such as teratogenicity or leukaemogenity. Nevertheless, we were able to find in the literature quite a few reports describing pregnant women who were affected with hematologic malignancies and receiving G-CSF, mainly as a supportive measure but also for the autologous mobilization of stem cells, while receiving chemotherapy in the second or third trimester (Reynoso et al., 1987, Reynoso and Huerta, 1994; Cavenagh et al., 1995; Lin et al., 1996; Claahsen et al., 1998; Achtari and Hohlfeld, 2000; Aviles and Neri, 2002; Siu et al., 2002). With the exceptions of the death of one fetus attributed to idarubicin (Reynoso and Huerta, 1994) and one child found to have a low intelligence quotient and malignancy (Reynoso et al., 1987), all the others had normal deliveries and healthy newborns (Table II). These reports are generally encouraging as no congenital malformations or other toxicities attributable to the CSFs, and mainly to G-CSF, have been observed; however, the data are still limited.

### G-CSF administration to healthy donors and leukemogenicity risk

The association of G-CSF administration with an increase in myeloid leukemia and/or myelodysplasia risk in patients with congenital neutropenia (Donadieu et al., 2005; Rosenberg et al., 2006), along with anecdotal reports of myeloid leukemia occurring in related PBSC donors (Bennett et al., 2006), has raised concerns about long-term risks of G-CSF administration to healthy donors (Table III). Small studies by Cavallaro et al. and Anderlini et al. in which normal stem cell donors were actively contacted after donation of PBSC (overall about 400 donors) have shown no late effects associated with short-term G-CSF therapy, with 3–6 years of follow-up (Cavallaro et al., 2000; Anderlini et al., 2002). Annual attempts at follow-up were also made for 2408 donors (median follow-up, 49 months; range, 2 days to 99 months) by the National Marrow Donor Program registry (Pulsipher et al., 2009). No cases of acute myelogenous leukemia or

### Table II G-CSF administration during pregnancy in women and fetal outcome.

<table>
<thead>
<tr>
<th>Study</th>
<th>Women with hematological malignancies, who received chemotherapy and G-CSF</th>
<th>Fetal outcome</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reynoso and Huerta (1994)</td>
<td>1</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
<tr>
<td>Lin et al. (1996)</td>
<td>1</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
<tr>
<td>Claahsen et al. (1998)</td>
<td>1</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
<tr>
<td>Reynoso and Huerta (1994)</td>
<td>1</td>
<td>Fetal death</td>
<td>After exposure to high dose Idarubicin &amp; Cytosine Arabinose</td>
</tr>
<tr>
<td>Achtari and Hohlfeld (2000)</td>
<td>1</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
<tr>
<td>Siu et al. (2002)</td>
<td>1</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
<tr>
<td>Reynoso et al. (1987)</td>
<td>6</td>
<td>Healthy newborn</td>
<td>1 with thyroid cancer at age 11 and neuroblastoma at age 14, but alive and healthy</td>
</tr>
<tr>
<td>Aviles and Neri (2001)</td>
<td>84</td>
<td>Healthy newborn</td>
<td>No late complications</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Donors (pregnant women) who received G-CSF</th>
<th>Leukemogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavallaro et al. (2000)</td>
<td>101</td>
<td>No evidence of increased risk</td>
</tr>
<tr>
<td>Anderlini et al. (2002)</td>
<td>281 (PBSC)</td>
<td>No evidence of increased risk</td>
</tr>
<tr>
<td>Pulsipher et al. (2009)</td>
<td>2408</td>
<td>No evidence of increased risk</td>
</tr>
<tr>
<td>Halter et al. (2009)</td>
<td>27,770 (BM harvest)</td>
<td>No evidence of increased risk</td>
</tr>
<tr>
<td>Halter et al. (2009)</td>
<td>23,254 (PBSC)</td>
<td>No evidence of increased risk</td>
</tr>
</tbody>
</table>

PBSC: peripheral blood stem cells; BM, bone marrow.
myelodysplasia were reported. Twenty-five non-hematologic cancers of various types occurred along with one case of chronic lymphocytic leukemia. Comparisons of the incidence of these cancers with the expected rates according to the Surveillance, Epidemiology and End Results database showed no evidence of increased cancer risk in the donor cohort.

However, a word of caution comes out of publications from Nagler et al., Shapira et al. and Kaplinsky et al., indicating that G-CSF administration to normal healthy donors results in temporary alterations in replication timing and DNA stability, which can result in chromosomal alterations and aneuploidy. These epigenetic and genetic alterations were observed in lymphocytes and other mature white blood cell subsets but not in purified CD34+ stem/progenitor cells (Nagler et al., 1999, 2004; Kaplinsky et al., 2003; Shapira et al., 2003). Although these laboratory observations may be a cause for concern, they did not demonstrate a definite link between G-CSF and leukemogenesis, and their significance is uncertain.

In addition to all the above, Halter et al. reported the development of hematologic malignancies in 8 among 7770 healthy bone marrow, I stem cell donors and in 12 among 23 254 PBSC donors: the higher incidence rate in peripheral blood donors is most likely explained by the fact that they were older than the bone marrow donors (Halter et al., 2009). In that report, although it was demonstrated that hematologic malignancies do occur in healthy donors, it was also emphasized that in both groups (healthy bone marrow and PBSC donors) the observed incidence rates of hematologic malignancies were below the age-specific crude incidence rates for a normal population (Halter et al., 2009). Nevertheless, a risk of promotion of a malignant myeloid clone cannot be excluded. Therefore, careful prospective tracking of both short- and long-term adverse events should be part of all studies involving G-CSF administration to normal donors and especially pregnant and lactating women. The safety and other issues related to G-CSF administration to healthy donors are beyond the scope of our current review and are extensively discussed in previous reviews (Pulsipher et al., 2006; Shaw et al., 2011).

Conclusions

Animal and human data on administration of G-CSF and GM-CSF during pregnancy suggest that there is no major risk for the embryo or fetus with regard to long-term effects, such as teratogenicity or leukemogenicity. Administration of CSFs should probably not be performed during the time of most active organogenesis (first trimester). After delivery and during lactation, there should be a delay of at least 3 days before breast feeding to allow for clearance of CSF from the mother’s milk.

To conclude, administration of CSFs to women during the second and third trimesters appears to be safe based on available data but the clinical experience is rather limited. Therefore, for both bone marrow and PBSC transplants, administration of CSF during pregnancy should not be used if other alternatives are available.

Acknowledgement

We would like to thank Prof Essie Kariv for her valuable and thoughtful comments and crucial help in editing the manuscript.

Authors’ roles

The initial idea and concept for the review was of A.N. who also helped in allocation of some of the relevant literature. All data and results were extracted by the first author (I.P.) and were crosschecked by two research specialists (A.N. and A.S.). The first author (I.P.) was responsible for the main writing of the study, and all authors drafted the paper and revised it critically.

Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

References


