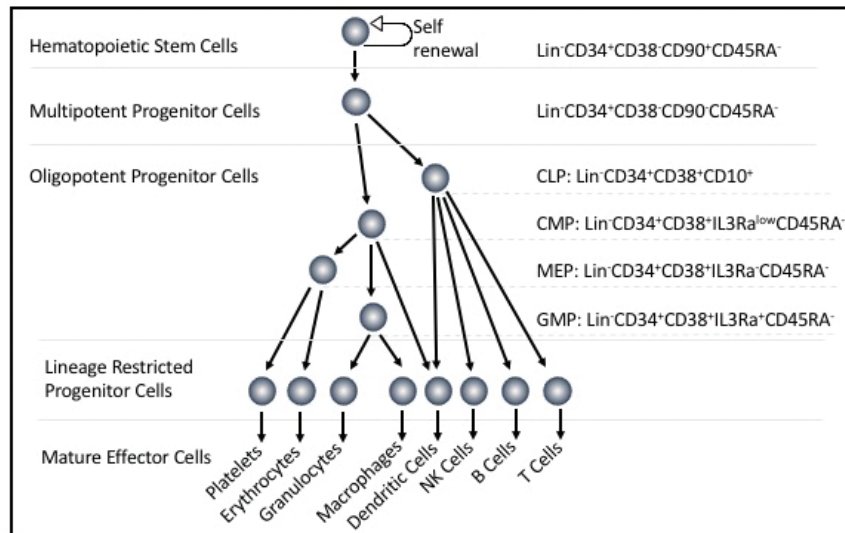


# Stem Cell Mobilization Protocols: Filgrastim vs. Mozobil

Lily C. Trajman, Ph.D.

## Introduction

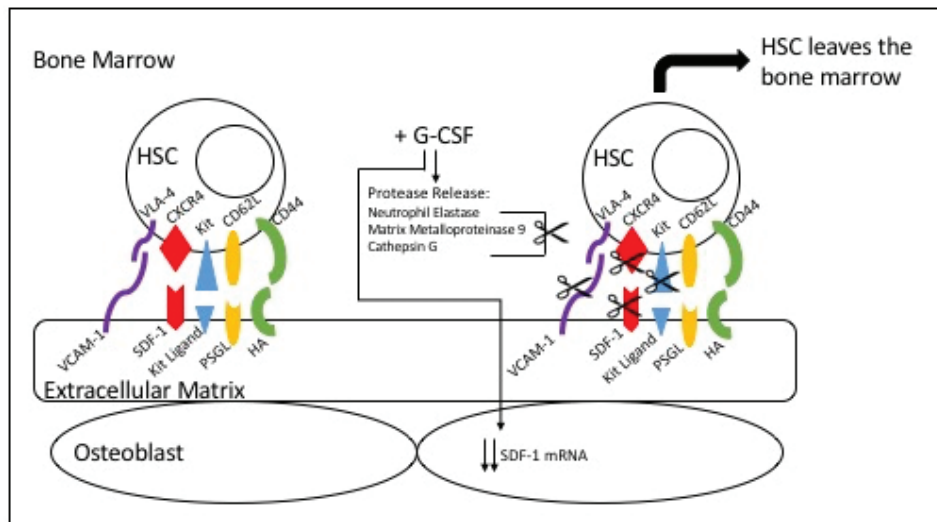
Hematopoietic stem cells (HSCs) are primitive cells capable of both self renewal and producing progenitor cells that can differentiate into all the cells of the hematopoietic system (Figure 1). Hematopoietic stem cell (HSC) transplantation is an increasingly common therapy used to treat a number of cancers, including leukemia, lymphoma, multiple myeloma, and solid tumors such as neuroblastomas and Ewing’s Sarcoma (Ali, 2015). It can also cure other types of diseases, such as sickle cell anemia,  $\beta$ -thalassemia, Fanconi’s Anemia and various immunodeficiency syndromes. Much research has focused on the efficient collection of hematopoietic stem cells for use in transplantation. HSCs can be found in three locations: cord blood, bone marrow (BM), and the peripheral blood (PB). Of these three options, cord blood is easily obtained but has a low total number of HSCs and its source does not allow multiple collections from the same donor. Collection of HSCs from the bone marrow is a surgical procedure requiring general anesthesia, but gives a large number of cells. Collection of PBSCs is relatively easy and repeatable, since it is not a surgical procedure, but steady state peripheral blood contains few HSCs.



**Figure 1:** Lineage markers on Hematopoietic stem and progenitor cells. CLP: Common Lymphoid Progenitor; CMP: Common Myeloid Progenitor; MEP: megakaryocyte-erythroid progenitor; GMP: granulocyte-macrophage progenitor.

There is no known cell surface protein unique to HSCs; instead, HSCs are usually identified by their elevated surface expression of the transmembrane glycoprotein CD34, which is expressed on both stem and progenitor cells (Figure 1). In bone marrow, between 1 and 3% of cells are CD34+; in peripheral blood, 0.1% of cells are CD34+ (Bender, 1994; Van Epps, 1994). A successful allogeneic transplant requires 2-5x10<sup>6</sup> CD34+ cells per kilogram of recipient body weight. Thus in order to acquire enough stem cells for an allogeneic transplant in a 70kg recipient, you would need to process between 50 and 350L of blood via apheresis to obtain a sufficient number of CD34+ cells. This is an unrealistic blood volume to process, even from multiple donors. It therefore becomes imperative to find a way to mobilize stem and progenitor cells to migrate from their niche in the bone marrow into the peripheral blood.

HSCs reside in a bone marrow microenvironment made up of stromal cells and an extracellular matrix rich in fibronectin, proteoglycans and collagen. They are retained in this microenvironment due to interactions between HSC surface proteins (notably c-kit, VLA-4, CXCR4, CD62-L and CD44) and their ligands found in the extracellular matrix of the bone marrow stroma (KL, VCAM-1, SDF-1, PSGL and HA respectively) (Nervi, 2006). Trafficking from the bone marrow requires migration through the vascular barrier into the circulatory system, and is accomplished by the downregulation and / or proteolytic cleavage of HSC cell surface receptors and their ligands. Specifically, c-kit, VLA-4, VCAM-1 and SDF-1 are subject to cleavage by proteases, while SDF-1 mRNA transcription in osteoblasts is also downregulated following G-CSF administration (Figure 2) (Nervi, 2006).



**Figure 2:** HSC homing in the bone marrow microenvironment. A variety of receptors expressed by HSCs bind to ligands in the extracellular matrix. Addition of G-CSF induces protease release that cleaves the extracellular portion of VCAM-1 and SDF-1 in the extracellular matrix, as well as CXCR4 and VLA-4 on the HSC. This allows trafficking of the cell out of the bone marrow. In addition, G-CSF causes downregulation of SDF-1 mRNA in osteoblasts.

Several different growth factors, cytokines and chemotherapeutic agents have been shown to induce HSC mobilization. Thus far, three different approaches have been used with success to mobilize stem cells in humans. Researchers initially noted an increase in PBSCs during the hematopoietic recovery phase after chemotherapy, making this a viable option for HSC collection for autologous stem cell transplants (Richman, 1976). Second, hematopoietic growth factors and cytokines such as granulocyte colony stimulating factor (G-CSF), IL-7, IL-8, IL-12, and granulocyte/macrophage colony stimulating factor (GM-CSF) have been used to mobilize HSCs from the bone marrow. Third,

mobilization has been prompted using Plerixafor (Mozobil), a small molecule antagonist that reversibly inhibits the interaction between CXCR4 and stromal-derived factor 1 $\alpha$ (SDF-1 $\alpha$ ). Blocking the binding of SDF-1 $\alpha$  allows HSCs to exit the bone marrow and traffic to the peripheral blood. Other small molecule inhibitors have also been tested (Nervi, 2006). The first two approaches offer higher PBSC collection numbers in healthy donors, since HSCs proliferate after treatment and also migrate to the peripheral blood at a greater rate. Concurrent use of Plerixafor and G-CSF has shown synergistic mobilization of HSCs in patients with multiple myeloma and lymphoma compared to G-CSF alone (Flomenberg, 2005; Callandra, 2008).

This paper will examine the use of recombinant human G-CSF (Filgrastim (Amgen)) and Mozobil (Genzyme) for CD34+ cell mobilization in healthy donors, including the mechanism of action, side effects, and therapy potential.

## Filgrastim (Amgen)

### Background

The growth factor G-CSF has long been recognized as an effective method for increasing HSC proliferation and mobilization, thereby increasing the number of PBSCs collected during apheresis. It is commonly used to mobilize HSC in healthy donors. Filgrastim is a G-CSF analog produced using recombinant DNA technology. In this case, the cDNA encoding human G-CSF was inserted into the Escheria coli genome, allowing high levels of expression and easy collection from the growth medium.

### Mechanism of action

Filgrastim and endogenous G-CSF function by downregulating the surface expression of SDF-1 $\alpha$  on osteoblasts, while at the same time releasing proteases from neutrophils and monocytes that cleave the adhesion molecules Kit, VCAM1 and CXCR4 found on bone marrow HSCs. The reduction in chemokine expression by stromal cells coupled with the proteolytic cleavage of homing receptors on the HSC surface releases HSCs into the blood, with the number of CD34+ cells in the blood peaking after 4-6 days of G-CSF administration (Nervi, 2006). G-CSF also acts directly on HSCs to promote proliferation (Figure 2).

Filgrastim is administered daily for at least four days at a concentration of 10 $\mu$ g/kg. Early studies showed that CD34+ cells peaked after 4-6 days of administration, with higher doses producing a greater number of CD34+ cells per mL up to a plateau at 10 $\mu$ g/kg (Grigg, 1995). Current protocols generally "pre-treat" donors with 10 $\mu$ g/kg G-CSF for four days, and the first round of apheresis occurs on day five.

### Dosing Schedule / Protocol

The recommended protocol for Neupogen is:

- 10 $\mu$ g/kg Neupogen administered subcutaneously on days 1-5, with apheresis beginning on day 5.
- Continued daily doses of Neupogen through day 7, to be discontinued if sufficient numbers of CD34+ cells are collected earlier.
- The FDA data sheet can be found here:

[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2013/103353s51571bl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/103353s51571bl.pdf)

## Other Advantages / Disadvantages

Side effects related to G-CSF are generally mild to moderate and transient in nature. In a 1996 experiment involving 102 normal donors, 90% of those who received G-CSF experienced one or more side effects. The most common side effect is acute bone pain (reported by 89% of donors), followed by headache (39%), body aches (23%), fatigue (14%) and nausea and/or vomiting (12%) (Stroncek, 1996). Similarly, a larger prospective study compiled in 2009 concluded that “complete recovery is universal” after looking at adverse effects among over 2400 donors, although it also noted that transient side effects were found in 85% of donors (Pulsifer, 2009). A far more rare but serious side effect is splenic rupture following G-CSF administration, which occurs with an incidence of 1 in 5,000-10,000 donors (Akyol, 2014; Falzetti, 1999).

## Side Effects

Administration of G-CSF to healthy donors has raised a number of safety concerns centered around the development of hematological malignancies following growth factor administration, especially since deregulation of genes and epigenetic changes have been noted following administration of G-CSF (Hernandez, 2007). An analysis of 2400 healthy donors found no cases of acute myelogenous leukemia or myelodysplasia at follow up appointments (median time to follow-up was 49 months); however there was one case of chronic lymphocytic leukemia and 25 reported cases of non-hematologic cancers. When compared to the incidence of various cancers in the general population there was no increase among donors who had received the G-CSF mobilization regimen (Pulsifer, 2009). This has been confirmed by various other prospective analyses in the US and Europe, although Stroncek and McCullough note that donor follow up studies must continue since malignancies induced due to G-CSF administration may not appear for 10 years or more (Stroncek, 2012).

Overall, G-CSF represents a safe and potent mechanism for mobilizing HSCs to the peripheral blood. Side effects are common but largely transient and only rarely severe enough to require hospitalization.

## Mozobil (Genzyme)

### Background

Plerixafor (marketed by Genzyme as Mozobil) is a small molecule inhibitor initially discovered in the context of HIV research, where it was found to inhibit HIV-1 and HIV-2 entry into cells in the 1-10nM concentration range. It is a potent and specific antagonist of the T cell co-receptor CXCR4 (used by T-lymphotrophic HIV strains to gain entry to the cell). During Phase I clinical trials an unexpected side effect of this drug was an observed increase in circulating white blood cells, specifically CD34+ hematopoietic stem and progenitor cells (DeClercq, 2009).

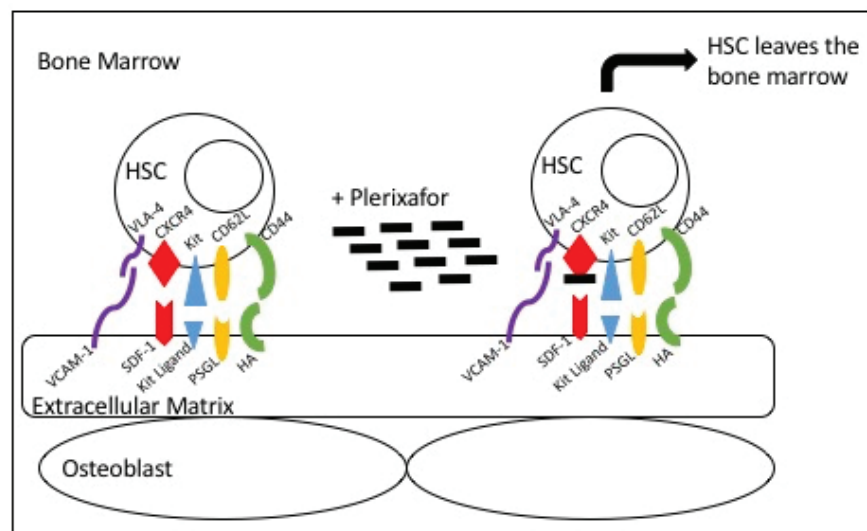
Mozobil can be used synergistically with Filgrastim to enhance HSC collection numbers; the combination of four or five days of G-CSF administration plus one dose of Mozobil results in a 2.5- to 3.8-fold increase in CD34+ cells collected during one apheresis session as compared to G-CSF alone (Gazitt, 2007; Liles, 2005). It is also often used as a “rescue” drug to enhance the collection of CD34+ cells used for autologous transplants in patients with Non-Hodgkin’s Lymphoma or Multiple Myeloma if the patient fails to mobilize sufficient numbers of cells with G-CSF alone.



## Mechanism of action

Plerixafor is a small molecule CXCR4 antagonist. The interaction between CXCR4, a chemokine receptor expressed on CD34+ HSCs and HPCs in the bone marrow, and Stromal cell derived factor 1 $\alpha$  (SDF-1 $\alpha$ ), a chemotactic cytokine produced by mesenchymal stromal cells in the bone marrow, causes CD34+ cells to be retained in the bone marrow microenvironment. Specifically, chemotaxis of CXCR4 toward SDF-1 helps HSCs home to the bone marrow; once there, the binding of CXCR4 to SDF-1 $\alpha$ , as well as the induction of other adhesion molecules, keeps HSCs localized to the bone marrow microenvironment. Plerixafor specifically and reversibly inhibits the binding of CXCR4 to SDF-1 in a concentration dependent manner, and inhibition of this interaction is sufficient to release HSCs into circulation (Figure 3).

Plerixafor has been shown to enhance peripheral CD34+ cell numbers in healthy donors when used alone or in conjunction with a G-CSF dosing regimen. Plerixafor increases peripheral CD34+ cell counts in a dose dependent manner, and the FDA has approved a dose of 0.24mg per kilogram actual weight. Following a single subcutaneous or intravenous injection of Plerixafor, an increase in circulating CD34+ cell numbers is evident within one hour, and CD34+ cell numbers peak at 9 to 10 hours post injection. Plerixafor has a half life of 3-6 hours in the plasma, and circulating CD34+ cell numbers return to baseline by 24h post-injection (Liles, 2003). If used in conjunction with G-CSF, a single dose of Plerixafor (0.24mg/kg) is administered on day four of G-CSF pretreatment, approximately 8-12 hours before apheresis begins (Steinberg, 2010). Synergistic use of Plerixafor and G-CSF increases the number of CD3+ cells per kg obtained during apheresis by 3.8-fold compared to either agent alone (Liles, 2005).



**Figure 3:** Trafficking of HSCs out of the bone marrow following Plerixafor administration. Plerixafor blocks the interaction between CXCR4 on the HSC and SDF-1 in the bone marrow extracellular matrix.

Currently Plerixafor is only approved by the FDA as a mobilizing agent when used in conjunction with G-CSF; however, initial studies indicate that Plerixafor alone can increase circulating CD34+ numbers to a similar degree as G-CSF. A subcutaneous injection of 0.24mg/kg Plerixafor results in a 10- to 15-fold increase in circulating CD34+ cells after 9 hours, comparable to the number of cells obtained after a five day dosing regimen with G-CSF (Liles, 2003).

## Dosing Schedule / Protocol

The FDA-approved mobilization regimen is as follows:

- 10 g/kg G-CSF administered in the mornings on day 1-4
- 0.24mg/kg Mozobil administered in the evening on day 4, approximately 11 hours prior to the start of apheresis.
- Continue Mozobil injections daily for up to 4 days.
- The full data sheet for Mozobil can be found here:

[http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2014/022311s015lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022311s015lbl.pdf)

## Side Effects

Most side effects were mild and transient. The most common side effects of Plerixafor injections are diarrhea (37%) and nausea (34%), followed by injection and infusion site reactions (34%), fatigue (27%) and headache (22%). Prospective analyses indicate that Plerixafor is a safe and effective mechanism for enhancing CD34+ cell collection by apheresis ([http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2014/022311s015lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/022311s015lbl.pdf)).

## Other Advantages / Disadvantages

Because Plerixafor and G-CSF function to increase the number of PBSCs via different mechanisms, there is some indication that the different mobilization regimes favor different progenitor cell populations. In macaques, dosing with Plerixafor enriches for B-, T- and Mast Cell precursors, while dosing with G-CSF enriches for neutrophil and mononuclear phagocyte precursors. Intriguingly, dosing with both Plerixafor and G-CSF together resulted in the upregulation of a different set of genes than dosing with each individual agent. Thus not only does administration of Plerixafor increase the number of PBSCs collected during apheresis, it may allow a more comprehensive representation of all stem and progenitor lineages found in the bone marrow (Donahue, 2009).

A comparison of mobilization with Plerixafor and G-CSF in humans has shown that mobilization with Plerixafor results in fewer CD34+ cells/kg and higher numbers of CD3+ and CD4+ cells/kg. Despite this, engraftment and reconstitution rates were comparable (Devine, 2008). Plerixafor appears to offer a faster protocol for CD34+ cell collection from the peripheral blood with fewer reported side effects than G-CSF.

## Conclusions

Apheresis represents a non-invasive and easily repeatable method for collecting PBSCs from healthy donors. Because of the low level of circulating PBSCs, various methods for increasing PBSC mobilization to the blood have been tested. Among healthy donors, administration of G-CSF and Plerixafor synergistically enhances the number of PBSCs recovered following apheresis, and either agent alone produces a similar elevation in PBSC numbers and results in similar rates of engraftment. Neither G-CSF nor Plerixafor have been shown to cause serious adverse side effects in humans with any frequency. However, transient, mild side effects such as bone pain and nausea are common. Prospective studies indicate that healthy donors have universally recovered from the donation process.

## References

- Akyol G, Pala C et al (2014). "A rare but severe complication of filgrastim in a healthy donor: splenic rupture." *Transfusion and Apheresis Science* 50(1): 53-55.
- Bender JG, Unverzagt K, Walker DE, Lee W, Smith S, Williams S, Van Epps DE (1994). "Phenotypic analysis and characterization of CD34+ cells from normal human bone marrow, cord blood, peripheral blood, and mobilized peripheral blood from patients undergoing autologous stem cell transplantation." *Clinical Immunology and Immunopathology* 70: 10-18.
- Callandra G, McCarty J, McGuirk J, et al (2008). "AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data." *Bone Marrow Transplant* 41(4):331-338.
- DeClercq E (2009). "The AMD3100 Story: The Path to the Discovery of a Stem Cell Mobilizer (Mozobil)." *Biochemical Pharmacology* 77(11): 1655-1664.
- Devine SM, Vig R, Rettig M et al (2008). "Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4-SDF-1 interaction." *Blood* 112: 990-998.
- Donahue RE, Jin P, Bonifacino AC et al (2009). "Plerixafor (AMD3100) and granulocyte colony stimulating factor mobilize different CD34+ cell populations based on global gene and microRNA expression signatures." *Blood* 114(12): 2530-2541.
- Falzetti F, Aversa F, Minelli O and Tabilio A (1999). "Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor". *Lancet* 353(9152), 555.
- Fiala MA, Park S et al (2016). "Remobilization of hematopoietic stem cells in healthy donors for allogeneic transplantation." *Transfusion* (online preview).
- Flomenberg N, Devine SM, Dipersio JF, et al The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone *Blood* 2005;106(5):1867-1874
- Gazitt Y, Freytes CO, Akay C, et al (2007). "Improved mobilization of peripheral blood CD34+ cells and dendritic cells by AMD3100 plus granulocyte colony stimulating factor in non-hodgkin's lymphoma patients." *Stem Cells Dev.* 2007 Aug;16(4):657-66.
- Grigg AP, Roberts AW, Raunow H et al (1995). "Optimizing dose and scheduling of Filgrastim (Granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers." *Blood* 86(12): 4437-4445.
- Hernandez JM, Castilla C, Gutierrez NC, et al. (2005). "Mobilisation with G-CSF in healthy donors promotes a high but temporal deregulation of genes." *Leukemia* 19: 1088-1091.
- Lack NA, Green B, Dale DC, Calandra GB, Lee H, MacFarland RT, Badel K, Liles WC, Bridger G (2005). "A pharmacokinetic-pharmacodynamic model for the mobilization of CD34+ hematopoietic progenitor cells by AMD3100." *Clin Pharmacol Ther* 77: 427-436.

Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, Dale DC (2003). "Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist." *Blood* 102: 2728–2730.

Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, Christensen J, Wood B, Price TH, Dale DC (2005). "Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist." *Transfusion* 45: 295–300.

Pulsipher MA, Chitphakdithai P, Miller JP et al (2009). "Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the national marrow donor program." *Blood* 113(15), 3604–3361.

Richman CM, Weiner RS, Yankee RA (1976). "Increase in circulating stem cells following chemotherapy in man." *Blood* 47(6): 1031-39.

Steinberg M and Silva M (2010). "Plerixafor: A Chemokine receptor-4 antagonist for mobilization of hematopoietic stem cells for transplantation after high dose therapy for non-hodgkin's lymphoma or multiple myeloma." *Clinical Therapeutics* 32(5): 821-843.

Stroncek DF, Clay Me, Petzoldt ML et al (1996). "Treatment of normal individuals with granulocyte-colony stimulating factor: donor experiences and the effects on peripheral blood CD345+ cell counts and on the collection of peripheral blood stem cells." *Transfusion* 36(7): 601-10.

Stroncek DF and McCullough J (2012). "Safeguarding the Long Term Health of Hematopoietic Donors: A Continuous and Evolving Process to Maintain Donor Safety and Trust." *Expert Review of Hematology* 5(1): 1-3.

Van Epps DE, Bender J, Lee W, Schilling M, Smith A, Smith S, Unverzagt K, Law P, Burgess J. "Harvesting, characterization, and culture of CD34+ cells from human bone marrow, peripheral blood, and cord blood." *Blood Cells* 20: 411-23.