

# The Use of Growth Factors in Hematopoietic Stem Cell Transplantation

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**Abstract:** Mobilized, peripheral blood stem cells (PBSC) are increasingly used for both autologous and allogeneic transplants. Granulocyte-colony-stimulating factor is the most widely used cytokine for mobilization. Several different mechanisms of stem cell mobilization have been proposed including protease-dependent and non-protease-dependent mechanisms. In autologous transplants, the addition of chemotherapy to mobilization can enhance the yield of PBSC collected but with substantial adverse effects, and not necessarily faster engraftment. In allogeneic transplants, the use of mobilized PBSC is associated with faster engraftment and donor chimerism compared to bone marrow. In the majority of studies, the rate of acute graft-versus-host disease (GVHD) has not been shown to be significantly higher with PBSC, but the rate of chronic GVHD appears to be increased. Several different strategies have been proposed for patients and donors who fail initial mobilization, including the use of novel agents. AMD3100 (Plerixafor) works by directly inhibiting the interaction between stromal cell-derived factor-1 and its receptor CXCR4, and mobilizes hematopoietic stem cells within hours. It is being studied alone or in conjunction with growth factors for PBSC mobilization in both autologous and allogeneic settings. Although the use of growth factors after PBSC transplantation results in faster neutrophil engraftment its impact on treatment-related mortality and survival does not appear significant. Here, we review the biology and methods of PBSC mobilization, the effect of growth factors on normal donors and the controversies of growth factor use in the post-transplant setting. We also review the data on novel agents for mobilization of stem cells.

**Key Words:** Stem cell transplantation, Mobilization, G-CSF, AMD3100.

## INTRODUCTION

The use of allogeneic bone marrow (BM) transplantation for treatment of hematological malignancies was first introduced in the late 1960s, after the discovery of the major histocompatibility complex [1-4]. Donor stem cells engraft in recipients conditioned with myeloablative chemotherapy and radiation, and provide these patients with rapid reconstitution of hematopoietic lineages. The use of autologous BM transplantation followed, with a similar rationale of "rescue" after myeloablative conditioning regimens. Subsequent studies reported an increased incidence of relapse in patients who received T cell depleted grafts or syngeneic grafts and reduced relapses in patients who developed acute or chronic Graft-versus-Host-Disease (GVHD) [5-7]. The data supported the notion that allogeneic transplantation is associated with a potent Graft-Versus-Tumor (GVT) effect.

The use of peripheral blood stem cells (PBSC) to reconstitute multilineage hematopoiesis after myeloablative conditioning and autologous transplantation was first introduced in the 1980s [8-11]. Initially, chemotherapy alone was used to mobilize stem cells from BM to the peripheral blood. After the discovery of both human granulocyte colony-stimulating factor (G-CSF) [12] and granulocyte-macrophage colony-stimulating factor (GM-CSF) [13], the use of growth factors became a standard and reproducible way of mobilizing human CD34<sup>+</sup> stem cells for autologous transplantation. Since then, multiple randomized studies have demonstrated many advantages of PBSC over BM for autologous transplantation including less invasive collection methods, reduced morbidity, and faster engraftment and immune reconstitution [14-16].

There was initial concern regarding the use of mobilized PBSC as a source of graft for allogeneic stem cell transplantation. This concern was based on the presence of a 10- 50 fold increase in the T cell content of the growth factor mobilized peripheral blood products, which could potentially lead to higher rates of acute and chronic GVHD. However, transplantation with mobilized PBSC was associated with faster engraftment and reduced infectious complications [17-19]. Growth factor mobilized PBSC are now the

preferred graft source for allogeneic transplantation in the majority of centers, both in related and unrelated stem cell transplant settings.

Although growth factors are frequently being administered after both autologous and allogeneic transplantation to accelerate engraftment and hematopoietic recovery, the impact of growth factors on the outcome of allogeneic PBSC and long term immune reconstitution remains controversial [20-22].

In this article we will review the biology and mechanisms of stem cell mobilization and the use of growth factors in the post-transplant period. We will present current approaches to mobilize stem cells for autologous transplantation, specifics of growth factor use in allogeneic transplantation and the effect of growth factors on normal donors. Controversies surrounding the use of growth factors in post-transplant setting will also be discussed. Finally, we will review the data on novel investigational agents which might soon be integrated into our standard treatment strategies. At this time, only G-CSF and GM-CSF are Food and Drug Administration (FDA) -approved for the mobilization of stem cells.

## BIOLOGY AND MECHANISMS OF STEM CELLS MOBILIZATION

Under the steady state, hematopoietic stem cells reside in the BM and circulate in the peripheral blood in very low numbers (0.01-0.05 % of peripheral blood cells). The number of circulating stem cells can be significantly increased by mobilizing them from the BM with cytokines and/or chemotherapy. After transplantation, these circulating stem cells are capable of homing to the BM and reconstituting multilineage hematopoiesis.

Hematopoietic stem cells in the BM exist in a highly organized three-dimensional microenvironment comprised of a diverse population of stromal cells, osteoblasts and osteoclasts, in an extracellular matrix rich in fibronectin, collagens and proteoglycans. Osteoblasts play a pivotal role in regulating hematopoietic stem cell function by providing signals that can either maintain stem cell quiescence or direct self renewal and differentiation [23,24]. To enter the circulation, stem cells must migrate through a vascular barrier composed of endothelial cells, a basement membrane and a layer of adventitial cells. A number of stem cell adhesion molecules are potentially involved in tethering stem cells to the BM microenvironment. These include CXC receptor 4 (CXCR4), leukocyte

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function-associated antigen-1 (LFA-1), very-late antigen-4 (VLA-4), tyrosine kinase receptor c-kit, surface glycoproteins CD44 and CD62L and Mac-1. The stroma of the BM expresses cognate ligands for these adhesion molecules including stromal cell-derived factor-1 (SDF-1), vascular cell adhesion molecule-1 (VCAM-1), kit ligand (KL), CD62 and hyaluronic acid (Fig. 1).

Disruption of the interactions between adhesion molecules and their ligands by cytokines is the basis for stem cell mobilization. Experiments in mice and non-human primates have demonstrated that the interaction between SDF-1 and its receptor CXCR4 is critical for the trafficking of stem cells in the BM, and that the interruption of these interactions plays a pivotal role in stem cell mobilization by G-CSF [23,25]. Expression of the SDF-1 receptor on BM stroma decreases significantly during G-CSF administration [24]. The exact mechanism by which the interaction between SDF-1 and CXCR4 is interrupted remains unclear. There is some evidence that G-CSF treatment leads to proteolytic inactivation of CXCR4 and degradation of SDF-1 by neutrophil elastase and cathepsin-G [26-28]. However, studies in mice deficient in these specific proteases still showed decreased expression of SDF-1 and CXCR4 and normal stem cell mobilization after administration of G-CSF suggesting other possible mechanisms [29-31]. Recent studies have shown that G-CSF induces significant down-regulation of the level of SDF-1 mRNA in BM stromal cells suggesting that G-CSF regulates SDF-1 expression at the mRNA level [24]. Other frequently studied interactions are those between VLA-4 and VCAM-1. Antibodies directed to VLA-4 or VCAM-1 lead to mobilization of stem cells and VCAM-1 expression in bone marrow decreases sharply during mobilization with G-CSF [32-34]. The role of specific proteases was hypothesized but experiments demonstrated that VCAM-1 cleavage was not necessary for stem cell mobilization [29]. A number of other adhesion molecules have been implicated in stem cell trafficking including c-kit, CD62L, CD44, P- and E-selectins [32,35-37]. With better understanding of the interactions between

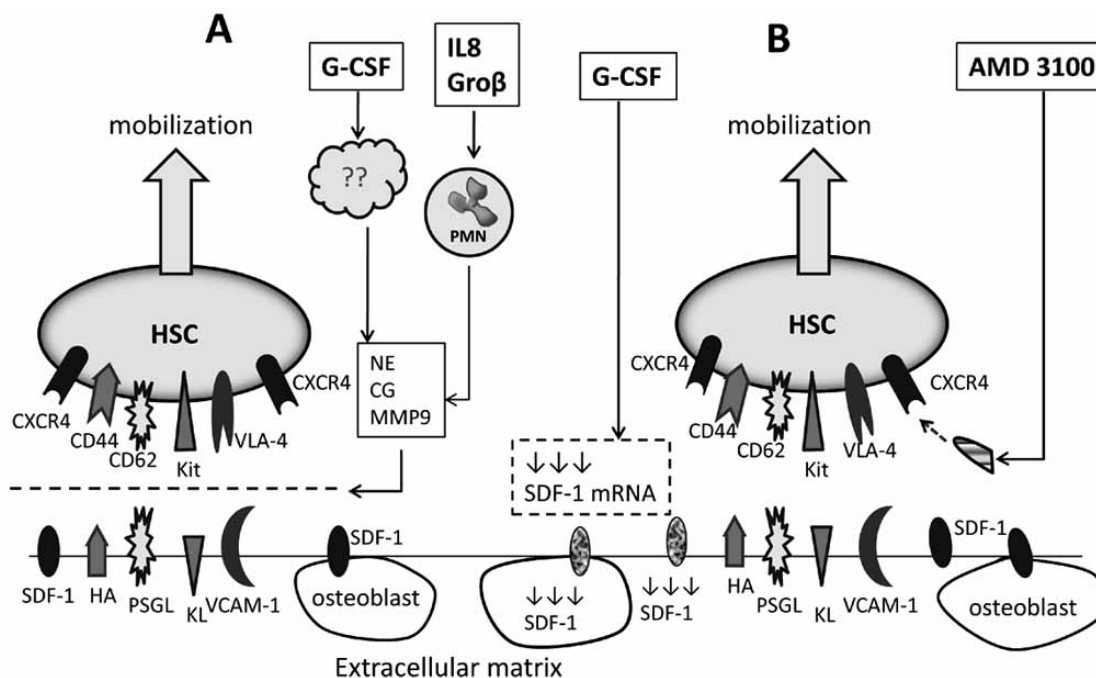
stem cells and the BM environment, specific targets for mobilization can be additionally explored.

### MOBILIZATION OF STEM CELLS FOR AUTOLOGOUS STEM CELL TRANSPLANTATION

As previously mentioned, multiple randomized trials have demonstrated the superiority of PBSC over BM for autologous stem cell transplantation. The benefits of PBSC include less invasive collection methods, reduced morbidity, faster engraftment, shorter hospitalization and lower total cost [14-16]. Initially, autologous PBSC transplants were performed using chemotherapy alone for the mobilization of stem cells. However, certain patients, particularly those heavily pretreated or with progressive disease and marrow involvement, failed to mobilize a sufficient number of stem cells. With the introduction of cytokines such as G-CSF and GM-CSF [12,13] the level of stem cells in the periphery increased up to 100 fold and their use, alone or after chemotherapy, have become the standard for mobilization of autologous stem cells.

### Minimum and Optimal CD34<sup>+</sup> Cell Yield for Autologous Stem Cell Transplantation

Several studies have examined the correlation between the number of CD34<sup>+</sup> cells infused and the time to engraftment, and attempted to determine the minimum CD34<sup>+</sup> cell dose needed for sustained engraftment after autologous transplantation [38-45]. While some groups [39,40,42-44] found the threshold for CD34<sup>+</sup> cells/kg to be  $1-3 \times 10^6$ , others suggested an optimal dose of  $> 5 \times 10^6$  CD34<sup>+</sup> cells/kg [38,41,45]. In these later studies neutrophil and platelet engraftment was faster and the need for other supportive measures (antibiotics, transfusion) was decreased. In summary, it appears that the infusion of  $2 \times 10^6$  CD34<sup>+</sup>/kg results in adequate engraftment, but a dose of  $> 5 \times 10^6$  CD34<sup>+</sup>/kg produces more rapid and predictable hematopoietic and specifically platelet recovery. It is, however, difficult to collect  $> 5 \times 10^6$  CD34<sup>+</sup>/kg in heavily pre-



**Fig. (1).** In the steady state hematopoietic stem cells (HSC) adhere to BM stroma through interactions between adhesion molecules on HSC and cognate ligands expressed on BM stroma and osteoblasts. **A:** Protease-dependent pathway of HSC mobilization. G-CSF, through an unknown mediator cell, and IL8 and GroB, through neutrophils and monocytes, induce the release of number of proteases (NE, CG, MMP9) into the BM environment resulting in proteolytic cleavage of key adhesion molecules. **B:** Non-protease dependent pathway. Recent studies in protease deficient mice suggest that non-proteolytic mechanisms may play a role in HSC mobilization. G-CSF induces transcriptional down-regulation of SDF-1 mRNA on BM stroma and osteoblasts. AMD 3100, the new and promising mobilization agent, reversibly and selectively blocks SDF-1 binding to CXCR4 resulting in rapid mobilization of HSC. Cathepsin G (CG), Chemokine receptor 4 (CXCR4), hyaluronic acid (HA), interleukin-8 (IL8), kit ligand (KL), matrix metalloproteinase-9 (MMP9), neutrophil elastase (NE), stromal cell derived factor-1 (SDF-1), vascular cell adhesion molecule-1 (VCAM-1), very late antigen-4 (VLA-4), P-selectin glycoprotein ligand-1 (PSGL).

treated patients. Although different sub-populations of CD34<sup>+</sup> cells (such as CD34<sup>+</sup>CD33<sup>-</sup> and CD34<sup>+</sup>CD38<sup>-</sup>) have been studied as possible predictors of hematopoietic engraftment, the total CD34<sup>+</sup> count is still the most commonly used indicator of adequate collection of stem cells [46-48].

### Cytokine-Induced Mobilization

G-CSF and GM-CSF are the only cytokines approved for mobilization of stem cells in the United States. A number of studies have compared stem cell mobilization with G-CSF and GM-CSF alone or in combination with chemotherapy [22,49-53]. G-CSF, as a single agent, mobilizes more CD34<sup>+</sup> cells than GM-CSF [51,52]. Combining G-CSF and GM-CSF does not improve the CD34<sup>+</sup> content in the harvest significantly [22,50]. However, for the patients who fail initial mobilization with G-CSF as a single agent, combination or sequential use of G-CSF and GM-CSF may be efficacious. The dose response to G-CSF and GM-CSF has been demonstrated, with higher doses resulting in increased yield of CD34<sup>+</sup> cells [38,54-57]. Different dosing schedules of growth factors were also evaluated (twice a day vs. once a day), but there is no evidence that any one schedule is superior [58-60] and donor/patient preference and convenience is a deciding factor. G-CSF is usually administered by subcutaneous injection at a dose of 10 µg/kg/day for 5 days [16,61]. Leukapheresis is then performed daily until the target number of stem cells is collected (usually for 1-5 days).

### Cytokines Plus Chemotherapy

Chemotherapy induces marrow aplasia. The addition of G-CSF enhances hematopoietic recovery and release of CD34<sup>+</sup> cells into the periphery. Although some small phase II trials reported an increase in the yield of CD34<sup>+</sup> cells collected after mobilization with chemotherapy plus growth factor comparing to growth factor alone, infusion of these PBSC products into autologous stem cell transplant recipients did not result in faster neutrophil or platelet recovery [62,63]. The optimal time for collection of stem cells after mobilization with chemotherapy plus G-CSF is 10-14 days as opposed to 4-5 days when G-CSF is used as single agent. Although mobilization with chemotherapy plus G-CSF generally requires fewer leukaphereses to collect an adequate number of stem cells [64,65], individual differences in response to chemotherapy, and the potential for complications result in more unpredictable collections that can delay the transplant and result in increased morbidity and cost. The use of chemotherapy as a part of mobilization is associated with significantly higher toxicities including increased risk for secondary malignancies, impairment of fertility, cardiac toxicity, hemorrhagic cystitis and anaphylactic reactions [64,66]. In addition, there is a higher risk of cytopenias and infections which may occasionally require hospital admission [63,64]. It was initially thought that chemotherapy might provide additional debulking of tumor prior to autologous transplant. However, studies in multiple myeloma (MM) and lymphoma showed that mobilization regimens that included chemotherapy did not reduce the risk of tumor contamination in the graft [67,68]. Additionally, Bourhis *et al.* found no difference in relapse rate in MM patients who received grafts contaminated with tumor cells comparing to those who received grafts without detectable tumor cells [69].

In conclusion, the literature indicates that the use of chemotherapy in addition to G-CSF for mobilization of stem cells carries no benefit for patient survival over use of G-CSF alone. A recent randomized trial comparing these two approaches found no differences in overall survival and progression-free survival after 21 months [62]. In addition, increased CD34<sup>+</sup> cells/kg obtained after chemotherapy plus G-CSF has not resulted in any significant enhancement of neutrophil or platelet engraftment [63].

### Remobilization

How to approach patients who failed to mobilize adequate number of stem cells ( $2 \times 10^6$  /kg) on the first attempt is complex

and still a matter of debate. Several factors are identified as predictors of poor mobilization including advanced age [70], amount of previous myelosuppressive chemotherapy and radiation [40,71,72], number of chemotherapy regimens [72,73], interval from last chemotherapy [68,74], refractory disease and a hypocellular marrow [72]. Although there is no specific age after which it is difficult or impossible to mobilize stem cells, the older the patient, the more difficult it is to mobilize an adequate yield of stem cells. The most important predictors of successful mobilization are the number of months since last chemotherapy or radiation and peripheral blood platelet count at the time of mobilization [70]. Patients with certain hematological malignancies such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL) and preleukemic syndromes are known to be poorly mobilizing patients. Several reports suggest an inverse relationship between the expression of adhesion molecules on stem cell surface and their ligands with adequate mobilization [50,75]. Strategies to manage poor mobilizers include dose escalation of G-CSF (12.5-50 µg/kg/day) [73], addition of another cytokine (GM-CSF, stem cell factor), mobilization with chemotherapy plus cytokine, harvesting cells directly from the BM, and the use of novel agents either alone or in combination with G-CSF.

### Potential Adverse Effects of Growth Factors

Although growth factors are generally considered safe, they are not without toxicities. Initial toxicity data came from studies of allogeneic donors [76]. Approximately 85% of recipients develop some somatic complaints, such as skeletal pain, fatigue, nausea and headache [76-80]. These are usually mild and rarely require discontinuation of the drug. Skeletal pain may start after the first injection and remain constant in many patients after two to three injections. Mild and transient alternations in electrolytes and liver enzymes have also been noted. Serious adverse effects are rare but have been described. Spontaneous splenic rupture was reported in several cases [81-85], precipitation of a sickle cell crisis in patients with sickle cell disease [86-88], increased autoimmune disease activity [87,89] and transient hypercoagulable states have been reported [90-93].

### MOBILIZATION OF STEM CELLS FOR ALLOGENEIC STEM CELL TRANSPLANTATION

PBSC have been increasingly used as a source of stem cells in allogeneic settings. Initial concern with use of PBSC for allogeneic transplantation was related to the possibility of increased risk of GVHD due to a 10-50 fold higher number of T cells in the graft compared to allogeneic BM. A number of trials compared the effect of mobilized PBSC vs. BM transplantation on the incidence of GVHD, relapse and survival [17-19,94-96]. The largest of those studies demonstrated no significant difference in overall survival between these two approaches, but faster engraftment with reduced infectious complications after PBSC transplants. The incidence of acute GVHD was similar with either stem cell source. However, the rate of chronic GVHD appeared to be less consistent, with some studies reporting increased incidence after PBSC and others observing no significant difference [94-96]. One large meta-analysis suggested an increased risk of chronic GVHD in those allogeneic transplant patients receiving G-CSF mobilized PBSC compared to BM transplantation [95].

G-CSF mobilized, allogeneic PBSC grafts contain 3-4 fold more CD34<sup>+</sup> cells compared to allogeneic BM grafts and are associated with faster engraftment and a shorter time to achieve complete donor chimerism when compared to BM. Similarly to autologous transplantation, the dose of CD34<sup>+</sup> cells infused correlates well with faster engraftment [97-100]. However, several studies have suggested that the higher doses of CD34<sup>+</sup> cells in the graft might increase the risk for developing GVHD. In the study by Prezepeiorca *et al.* higher CD34<sup>+</sup> cell dose was associated with an increased risk of acute GVHD [101] while other investigators did not confirm this correlation [102,103]. CD34<sup>+</sup> cell dose above  $8 \times$

$10^6$ /kg appeared to be associated with an increased risk of chronic GVHD [101,103-105]. Data from Fred Hutchinson Cancer Research Center suggested that unrelated donor transplants with low ( $< 2.7 \times 10^6$ ) and very high ( $> 10 \times 10^6$ ) doses of CD34<sup>+</sup> cells in the product were associated with worse overall survival [106]. Higher numbers of CD34<sup>+</sup> cells may be associated with improved survival in related PBSC transplants [107].

Similar to autologous transplants, the goal is to collect at least  $2 \times 10^6$  CD34<sup>+</sup> cells/kg, although the absolute minimum threshold has not been defined. In the majority of centers, donors receive 10  $\mu$ g/kg/day of G-CSF subcutaneously (sc) for five days followed by daily leukapheresis starting on day four or five until a target number of CD34<sup>+</sup> cells is collected.

The impact of allogeneic PBSC vs. BM graft on immune reconstitution is not well understood. Similar rates of acute GVHD are seen after infusion of BM or G-CSF mobilized PBSC in the allogeneic setting. This occurs despite a significantly higher number of CD3<sup>+</sup> cells infused with PBSC product. Cytokines produced by T cells, thought to play a role in the pathophysiology of GVHD, can be separated into two types. Type-1 (Th1) cytokines are considered to be pro-inflammatory, mediating GVHD, and Type-2 (Th2) cytokines are anti-inflammatory and inhibit development and activity GVHD. Studies in animals demonstrated that G-CSF mobilization polarizes the T cell response towards Th2 type cytokines and induces plasmacytoid differentiation of donor dendritic cells (DC2), potentially explaining the lower than expected rates of GVHD with PBSC [108-111].

#### GM-CSF

Several studies demonstrated that, used as a single agent, GM-CSF was less effective than G-CSF for mobilization of normal donors [48,112,113]. CD34<sup>+</sup> cell yield was lower after mobilization with GM-CSF and donors required more leukapheresis for an adequate collection. Although there appeared to be a dose-response effect with GM-CSF, there was more toxicity associated with higher doses (15  $\mu$ g/kg/day). Devine *et al.* [49] conducted a retrospective study reviewing the results of allogeneic PBSC transplants using different growth factors for mobilization. They showed that although the total dose of CD34<sup>+</sup> cell in the graft was significantly lower following GM-CSF compared to G-CSF, the engraftment was not compromised and hematopoietic recovery was similar. Product mobilized by GM-CSF contained fewer CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> and NK cells, and patients receiving those grafts experience a lower incidence of acute GVHD. These products also contained an increased amount of CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory T cells (known as Tregs) presumably associated with a reduction in risk for GVHD [114-117]. Randomized studies are needed to further evaluate for qualitative and quantitative differences in PBSC mobilized by different growth factors and their impact on engraftment, survival and GVHD.

#### Effect of Growth Factors on Normal Donors

Although allogeneic donors are generally considered "healthy", there are variations in the number of stem cells collected from different donors, and some of them are considered "poor mobilizers". At Washington University in St. Louis (unpublished data) normal donors who failed to mobilize minimum of  $1 \times 10^6$  CD34<sup>+</sup> cells/kg after 3 collections then underwent BM harvest resulting in yields of  $< 1 \times 10^6$  CD34<sup>+</sup> cells/kg suggesting that the reason for poor mobilization was low stem cell reserve. Retrospective analyses from IBMTR/EBMT (International and European Bone Marrow Transplant Registries) showed that 60 % of donors required more than one leukapheresis, and 15% required 3 or more leukaphereses to collect the target dose of CD34<sup>+</sup> cells [118]. Others had reported somewhat different data where 50-70 % of donors were successfully harvested with only one collection [97,119,120]. Donor age, steady-state CD34<sup>+</sup> count and dose of G-CSF were the most impor-

tant predictors for mobilization [121-123]. The mobilization process is generally well tolerated and the vast majority of donors complete it without difficulty. However, there are some potential adverse effects associated with growth factor administration. As previously mentioned, most common are mild and transient somatic complaints rarely requiring discontinuation of the drug. More serious potential toxicities include transient hypercoagulable state [90-92] or provoking an acute sickle-cell crisis [86,88]. People with coronary artery disease, cerebrovascular comorbidities and sickle cell disease/trait are excluded as donors. G-CSF can also precipitate autoimmune disease activity and the use of donors with these disorders is discouraged [87,124-126]. Several studies have shown transient splenic enlargement in G-CSF recipients and there have been five reported cases of spontaneous splenic rupture [81-85]. All of these donors fully recovered either spontaneously or after surgical intervention. In a retrospective analysis of 1448 donors from the IBMTR/EBMT registry, G-CSF was used for mobilization in >99% of cases, 20% of normal donors required placement of central venous catheter for leukapheresis, 85% were collected with one or two leukapheresis procedures, with only 1% experiencing adverse events, and no fatalities [118].

One potential concern associated with growth factor administration to normal donors is the effect of growth factor itself on the donor hematopoietic system. Several studies suggested that even short term administration of G-CSF might induce some alterations in genomic stability. There is speculation that a G-CSF "challenge" might unmask and activate mutated recessive genes. These events could theoretically present a risk for siblings of patients with hematological malignancies who might themselves have a predisposition for certain malignancies [127,128], however, no clear causative link has been established. There is a need to define guidelines for prospective follow-up of related and unrelated donors mobilized with growth factors.

#### ROLE OF GROWTH FACTORS SUPPORT IN THE POST-TRANSPLANT PERIOD

The use of growth factors after BM transplantation to enhance hematopoietic recovery has been well established [129-131]. However, PBSC transplantation is associated with a larger number of infused progenitor cells commonly resulting in rapid hematopoietic recovery [20-22,129,132]. Investigators hypothesized that the additional administration of growth factors after PBSC transplants would not significantly accelerate engraftment. Also, there was a concern that growth factors after allogeneic PBSC transplantation might increase the incidence of acute GVHD [20,21]. G-CSF has several immunomodulatory effects on hematopoiesis that could potentially influence the outcome of allogeneic transplantation. These include alteration in antigen-presenting cell function and T cell reactivity, a shift toward Th2 type immune response, impaired production of IL-12, and decreasing IFN $\gamma$  and IL-4 production [109,133-135].

A recent meta-analysis of prophylactic use of G-CSF after autologous and allogeneic transplants found reduced risk of infection and decreased duration of parenteral antibiotics, with faster neutrophil recovery and shorter hospital stay but no significant difference in infection-related or treatment-related mortality [136]. There was no difference in the incidence of acute or chronic GVHD in the allogeneic setting. Several other studies have supported these findings [137-140]. Bishop *et al.* conducted a prospective randomized trial of G-CSF after allogeneic transplantation and found that patients receiving G-CSF had significantly faster neutrophil recovery. Platelet recovery appeared faster in the G-CSF group but the results were not statistically significant [141]. Regarding CD34<sup>+</sup> cell dose, a positive effect on neutrophil engraftment was seen at all dose levels but was less pronounced with higher doses of G-CSF. This trial found no increased incidence or severity of acute GVHD in those patients receiving G-CSF.

Khoury *et al.* performed a retrospective analysis of the IBMTR database to evaluate the impact of growth factor administration after allogeneic transplant for acute myelogenous and chronic myelogenous leukemia on the outcomes after transplantation [142]. The recipients of related BM, unrelated BM and related PBSC were followed for a median time of 30 months. Patients were stratified into those who started growth factors within seven days of transplant, after seven days, or not at all. All patients received myeloablative conditioning, and methotrexate and cyclosporine as a part of GVHD prophylaxis. Growth factors did shorten the neutropenic period, but without altering treatment related mortality at days +30 and +100. There was no difference in the incidence of acute or chronic GVHD, leukemia-free survival or overall survival.

In contrast, some retrospective studies have suggested increased incidence of acute GVHD and treatment-related mortality among G-CSF recipients [143-145]. A study from EMBT observed that when BM was the stem cell source the use of post-transplant G-CSF results in an increased incidence of acute and chronic GVHD, increased treatment related mortality, and lower disease-free and overall survival [145]. Similarly to other retrospective studies, G-CSF shortened the period of neutropenia but prolonged the period of thrombocytopenia after both PBSC and BM transplants. Similar data has been seen in patients who receive G-CSF after the infusion of marginal doses of CD34<sup>+</sup> in the autologous transplant setting [146]. The optimal time for initiation of growth factor administration after transplant is not well defined. Several studies suggest that there is no significant difference in the engraftment kinetics with early (day 0 or +1) vs. late (day +5) initiation of growth factors, while lower costs are associated with delayed administration [147-151]. In a prospective, randomized trial using different doses of G-CSF (5, 10 and 16 µg/kg/day) Bolwell *et al.* demonstrated that the dose of 5 µg/kg/day results in a similar rate of neutrophil engraftment, faster rate of platelet recovery and significantly less cost when compared with higher doses [152].

The reasons for these contrasting results are unclear and likely multifactorial. There are many variables potentially influencing hematopoietic recovery, such as quantity of progenitor cells infused, type of conditioning regimen, GVHD prophylaxis, diagnosis, disease status and previous therapies. Until recently, the diagnostic criteria for chronic GVHD have been vague and varied significantly among different centers [153]. Also, statistical methods used for analysis frequently differ among these trials. To further address the impact of G-CSF on immune reconstitution, GVHD and survival as well as the optimal time for its administration, randomized trials are needed. To test the hypothesis that PBSC transplantation leads to similar survival compared to BM transplantation, a phase III, randomized multicenter prospective trial was recently opened. It compares G-CSF mobilized PBSC with BM transplantation from human leukocyte antigen (HLA)-matched (5/6 or 6/6) unrelated donors for patients with a history of acute leukemia, myelodysplasia, chronic myelogenous leukemia or other myeloproliferative diseases. The primary objective of this trial is to compare two-year survival probabilities between those two groups. Other endpoints include the engraftment rate, incidence of graft failure, acute and chronic GVHD, time to discontinuation of immunosuppression, relapse rate, infection rate, adverse events, immune reconstitution and quality of life. In this trial, patients receive one of the four predefined conditioning regimens while GVHD prophylaxis is left at the discretion of transplant physician. [www.clinicaltrialsnet.org](http://www.clinicaltrialsnet.org)

## OTHER APPROACHES AND EXPERIMENTAL AGENTS

Some patients and donors fail to mobilize adequate number of stem cells with standard approaches and alternative mobilization strategies are needed. Several new cytokines and chemokines have emerged as potential agents for stem cell mobilization, including stem cell factor (SCF), IL-8, FLT3 ligand, Groβ and others.

AMD3100 is new promising agent for stem cell mobilization in phase III trials.

### AMD3100 (Plerixafor)

AMD3100 is a bicyclam derivate that specifically and reversibly inhibits binding of SDF-1 to its receptor CXCR4 leading to stem cell mobilization. It was initially developed as a potential treatment for HIV, blocking the HIV entry into CD4<sup>+</sup> T cell by binding to the HIV co-receptor CXCR4 [154]. Studies in healthy volunteers showed good tolerance with minimal and reversible adverse effects [155]. In these early studies it was noted that a single intravenous dose of AMD3100 caused leukocytosis in treated subjects. Bioavailability after sc injection was 87% with a dose dependent increase in CD34<sup>+</sup> cells in the peripheral blood [156,157]. A single dose of AMD3100 at 160-240 µg/kg sc resulted in 6-10 fold increase in CD34<sup>+</sup> cell count starting 1 hour, peaking at 9 hours after injection and declining to baseline within 24 hours [156,158]. Most frequently noted adverse effects were transient pain and injection site erythema, headache, paresthesias, diarrhea, bloating and nausea.

A phase I study by Devine *et al.* [159], assessing the safety and clinical effects of AMD3100 in patients with MM and NHL, showed sevenfold increase in circulating CD34<sup>+</sup> cells 6 hours after a single dose of AMD3100 at 240 µg/kg sc, demonstrating that AMD3100 is effective in chemotherapy pretreated patients. Cashen *et al.*<sup>1,2</sup> reported successful mobilization of stem cells with G-CSF plus AMD3100 in heavily pretreated patients with HL who are considered poor mobilizers.

Flomenberg *et al.* [160] compared G-CSF and G-CSF plus AMD3100 for mobilization of stem cells in patients with MM and NHL. All the patients underwent two mobilizations, one using G-CSF as a single agent and another using G-CFS plus AMD3100. Patients receiving combination of two agents mobilized more CD34<sup>+</sup> cells per leukapheresis, underwent fewer leukaphereses and had a higher yield of total CD34<sup>+</sup> cells collected. After mobilization with G-CSF plus AMD3100, 56% of recipients reached a minimum of 2 x 10<sup>6</sup> CD34<sup>+</sup> cells/kg after one collection and 100% of recipients after two collections.

Based on the results of autologous transplantation [161], AMD3100 is being tested for mobilization of stem cells in the allogeneic setting. Devine *et al.*<sup>3</sup> performed a pilot study assessing feasibility and safety of CD34<sup>+</sup> cells mobilized with AMD3100 alone for allogeneic transplantation. Normal sibling donors were mobilized using AMD3100 at the dose of 240 µg/kg sc 4 hours prior to leukapheresis. A week later, each donor underwent another mobilization using G-CSF alone at standard dose. The G-CSF mobilized product was kept as a backup. Seven of eight donors mobilized with AMD3100 collected at least 2 x 10<sup>6</sup> CD34<sup>+</sup> cells/kg. There were no significant toxicities associated with AMD3100 administration. With median follow up of 200 days, no significant increase in GVHD was noted in comparison to historic experience after transplantation of G-CSF mobilized PBSC.

Allogeneic PBSC mobilized with AMD3100 contain lower numbers of CD34<sup>+</sup> cells and significantly more T, B and NK cells compared to PBSC mobilized with G-CSF. There was initial concern that grafts mobilized by AMD3100 might demonstrate poor

<sup>1</sup> Cashen A, Devine S, Vij R, DiPersio J. AMD3100 + G-CSF improves hematopoietic progenitor cell (HPC) collection in patients with Hodgkin's disease (HD). *Blood* 2005; 106: 299.

<sup>2</sup> Cashen A, Calandra G, MacFarland RT, Lopez S, DiPersio J. A mobilizing regimen of AMD3100 and G-CSF increases stem cell collection in patients with Hodgkin disease, and PK is similar to that of non-cancer patients. *Blood* 2006; 108: 869.

<sup>3</sup> Devine S, Andritsos L, Todt L, Vij R, Bonde J, Hess D, *et al.* A pilot study evaluating the safety and efficacy of AMD3100 for the mobilization and transplantation of HLA-matched sibling donor hematopoietic stem cells in patients with advanced hematological malignancies. *Blood (ASH Annual Meeting Abstracts)* 2005; 106: 299.

engraftment and altered T cell function. Devine *et al.* demonstrated, using a mouse model, that mobilization with AMD3100, alone or with G-CSF, results in timely and stable engraftment and rates of GVHD similar to those after G-CSF.<sup>4</sup>

Two phase III, multicenter, randomized, double-blind, placebo-controlled studies compared AMD3100 plus G-CSF with G-CSF alone for mobilization of stem cells in patients with MM and NHL.<sup>5,6</sup> Patients received G-CSF at 10 µg/kg/day sc for 4 days and on the evening of the fourth day they received either AMD3100 at 240 µg/kg sc or placebo ("study drugs"). The leukaphereses were started on day 5, after the morning dose of G-CSF, and continued until CD34<sup>+</sup> was  $\geq 5 \times 10^6$ /kg (NHL) or  $\geq 6 \times 10^6$ /kg (MM) or total of 4 leukaphereses. Patients continued receiving their morning dose of G-CSF and evening dose of study drug until collection was completed. Patients who failed to collect  $\geq 2 \times 10^6$  CD34<sup>+</sup> cells/kg were eligible for rescue with AMD3100 plus G-CSF. These studies confirmed that the addition of AMD3100 to G-CSF is generally safe and well tolerated. Patients mobilized with AMD3100 plus G-CSF were more likely to achieve a target CD34<sup>+</sup> cell count with less leukaphereses and had successful transplant.

Mobilization of stem cells with AMD3100 as a single agent would result in shorter mobilization times compared with G-CSF (one day vs. four) and would avoid G-CSF associated adverse effects. Although, the yield of CD34<sup>+</sup> cells collected after mobilization with AMD3100 alone is lower than after G-CSF mobilization, adequate numbers are achieved. AMD3100 is currently being tested as a single agent for stem cell mobilization in MM patients. In a study by Flomenberg *et al.*, patients received AMD3100 at 240 µg/kg sc followed by leukapheresis 6 hours later. AMD3100 alone mobilized an adequate number of stem cells and provided fast and durable engraftment.<sup>7</sup>

Mobilization and homing of both, hematopoietic stem cells and cancer cells, involve similar signaling pathways [162]. There is concern that AMD3100 might mobilize malignant cells concurrently with CD34<sup>+</sup> cells subsequently increasing rates of recurrence. However, clinical trials to date have not observed significant increase in tumor cell content in PBSC products mobilized by AMD3100.

In preclinical studies using a leukemia mouse model, Nervi *et al.* demonstrated rapid mobilization of leukemia cells into the periphery.<sup>8,9</sup> Administration of CXCR4 and VLA-4 antagonists together had a synergistic effect on rapid mobilization of both hematopoietic progenitors and leukemia cells.<sup>9</sup> These results may have important implications on the development of new approaches for

the treatment of acute leukemia since circulating leukemia cells appear more sensitive to chemotherapy. There is an ongoing trial at Washington University in St. Louis using AMD3100 as an adjunct to chemotherapy in patients with relapsed or refractory acute myelogenous leukemia.<sup>10</sup>

Mobilization with AMD3100 as a single agent is appealing and could result in shorter and predictable mobilization without the side effects associated with G-CSF. Further trials are needed to determine whether AMD3100 should be used for all patients or only for those who are considered high-risk for mobilization failure. Longer follow-up is needed to ascertain durability of engraftment, incidence of GVHD and safety for normal donors. AMD3100 has been shown to be an effective and rapid mobilizing agent in MM and NHL patients in the two large randomized trials described above.

#### Stem Cell Factor (SCF)

Recombinant human SCF is a cytokine that acts on primitive multilineage hematopoietic cells and stimulates mobilization of myeloid, erythroid and lymphoid progenitors. In the majority of trials assessing its role in mobilization of PBSC in the autologous setting, SCF acts synergistically with G-CSF, significantly reducing number of leukaphereses required to collect the target CD34<sup>+</sup> cell count and increasing CD34<sup>+</sup> cells collected [163-165]. However, severe anaphylactoid reaction occurs in 5-10% of patients receiving SCF [166] and thus it has not been FDA-approved in USA. It is approved for use in Canada and New Zealand.

#### Gro-β/SB-251353

Gro-β is a chemokine which exerts its biological activity by binding to the CXCR2 receptor. SB-251353 is a recombinant, N-terminal truncated form of human Gro-β which binds to CXCR2 receptor with greater potency than the full length form of Gro-β. In murine and non-primate models it induces rapid mobilization of stem cells and acts synergistically with G-CSF [167-169]. A single injection of SB-251353 plus four days of G-CSF can increase the yield of CD34<sup>+</sup> cells collected fivefold. No clinical trials have been performed as yet.

#### Pegfilgrastim

Pegylated G-CSF has a prolonged half-life and is generally used to shorten chemotherapy-related neutropenia. In those patients, administration of pegfilgrastim resulted in mobilization of CD34<sup>+</sup> cells to peripheral blood [170]. Similar effect was observed with administration of pegfilgrastim to normal donors [171]. In a small study of patients with MM, successful mobilization of CD34<sup>+</sup> cells was obtained with cyclophosphamide followed by pegfilgrastim [172]. Since it shares a similar mobilization pathway with G-CSF and a similar toxicity profile, it is unlikely that it will be used in patients who fail previous mobilization with G-CSF.

#### hrPTH

Osteoblasts are known to play an important role in stem cell function and homeostasis through their physical interaction with trabecular bone and stem cells ("osteoblastic niche") and by secretion of cytokines that regulate hematopoiesis [173,174]. Parathyroid hormone (PTH) activates its receptors on osteoblasts and stimulates trabecular bone growth, thereby increasing osteoblastic niche [175]. The role of hrPTH to both mobilize stem cells and expand the BM niche remains unclear. Further pre-clinical and clinical trials will be necessary to determine its role in stem cell transplantation.

#### CONCLUSION

Mobilized PBSC, compared with BM harvest, has become the preferred source of hematopoietic stem cells for autologous and

<sup>4</sup> Devine S, Liu F, Holt M, DiPersio J. AMD3100- mobilized murine hematopoietic stem cells and T-lymphocytes have identical capacity to induce multilineage stem cell engraftment, donor T-cell chimerism and GVHD in mice compared with G-CSF mobilized cells. *Blood* 2003; 102: 938.

<sup>5</sup> DiPersio J, Micallef I, Stiff P, Bolwell B, Maziarsz R, Angell J, *et al.* A phase III, multicenter, randomized, double-blind, placebo controlled, comparative trial of AMD3100 (plerixafor)+G-CSF vs. placebo + G-CSF in non-Hodgkins lymphoma (NHL) patients for autologous hematopoietic stem cell (aHSC) transplantation. *Blood* 2007; 110: 601.

<sup>6</sup> DiPersio J, Stadtmauer E, Nademanee A, Stiff P, Micallef I, Angell J, *et al.* A phase III, multicenter, randomized, double-blind, placebo-controlled, comparative trial of AMD3100 (plerixafor)+G-CSF vs. G-CSF + placebo for mobilization in multiple myeloma (MM) patients for autologous hematopoietic stem cell (aHSC) transplantation. *Blood* 2008; 110: 445.

<sup>7</sup> Flomenberg N, Comenzo R, Badel K, Calandra G. Single agent AMD3100 mobilization of peripheral blood progenitor cells for autologous transplantation in patients with multiple myeloma (MM). *Blood* 2006; 108: 3381.

<sup>8</sup> Nervi B, Ramirez P, Holt M, DiPersio J. CXCR4/SDF-1 is a key regulator for leukemia migration and homing to the BM: impact of AMD 3100 on the *in vivo* response to chemotherapy. *Blood* 2006; 108: 569.

<sup>9</sup> Ramirez P, Holt M, Rettig MP, Ritchey JK, DiPersio J. Mobilization of normal mouse progenitors and acute promyelocytic leukemia (APL) cells with inhibitors of CXCR4 and VLA-4 in splenectomized and unsplenectomized mice. *Blood* 2007; 110: 2219.

<sup>10</sup> Uy G, Rettig MP, Ramirez P, Nervi B, Abboud CN, DiPersio JF. Kinetics of human and murine mobilization of acute myeloid leukemia in response to AMD3100. *Blood* 2007; 110: 867.

allogeneic transplantation. However, our current mobilization regimens are associated with various limitations and require improvement. In the last ten years there have been major advances in our understanding of the interactions between stem cells and BM microenvironment. Although proteases are known to play an important role in stem cell mobilization by disrupting interactions between various adhesion molecules, non-proteolytic mechanisms appear to be involved. With a better understanding of the pathways involved and recognition of specific targets, new agents for stem cell mobilization are being developed. AMD3100, a direct inhibitor of CXCR4, is one such novel agent being studied alone or in the conjunction with growth factors for stem cell mobilization. The use of growth factors following stem cell transplantation results in faster neutrophil recovery. However, randomized trials are needed to further determine the effects of growth factors on immune reconstitution, the incidence of acute and chronic GVHD, and associated mortality.

#### ABBREVIATIONS

BM	=	Bone marrow
CXCR4	=	Chemokine receptor-4
FDA	=	Food and drug administration agency
G-CSF	=	Granulocyte-colony-stimulating factor
GM-CSF	=	Granulocyte-macrophage colony-stimulating factors
GVHD	=	Graft-versus-host disease
GVT	=	Graft-versus-tumor
HL	=	Hodgkin lymphoma
KL	=	Kit ligand
LFA-1	=	Leukocyte function-associated antigen-1
MM	=	Multiple Myeloma
NHL	=	Non-Hodgkin lymphoma
PBSC	=	Peripheral blood stem cells
PTH	=	Parathyroid hormone
SCF	=	Stem cell factor
SDF-1	=	Stromal cell-derived factor 1
SC	=	Subcutaneously
VCAM-1	=	Vascular cell adhesion molecule-1
VLA4	=	Very late antigen-4

#### REFERENCES

- Thomas ED, Lochte HL Jr, Cannon JH, Sahler OD, Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. *J Clin Invest* 1959; 38: 1709-16.
- Buckner CD, Epstein RB, Rudolph RH, Clift RA, Storb R, Thomas ED. Allogeneic marrow engraftment following whole body irradiation in a patient with leukemia. *Blood* 1970; 35: 741-50.
- Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 1968; 2: 1366-9.
- Thomas ED, Bryant JI, Buckner CD, Clift RA, Fefer A, Fialkow PJ, *et al.* Allogeneic marrow grafting using HL-A matched donor-recipient sibling pairs. *Trans Assoc Am Physicians* 1971; 84: 248-61.
- Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; 75: 555-62.
- Weiden PL, Flournoy N, Sanders JE, Sullivan KM, Thomas ED. Antileukemic effect of graft-versus-host disease contributes to improved survival after allogeneic marrow transplantation. *Transplant Proc* 1981; 13: 248-51.
- Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, *et al.* Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; 300: 1068-73.
- Abrams RA, Glaubiger D, Appelbaum FR, Deisseroth AB. Result of attempted hematopoietic reconstitution using isologous, peripheral blood mononuclear cells: a case report. *Blood* 1980; 56: 516-20.
- Kessinger A, Armitage JO, Landmark JD, Weisenburger DD. Reconstitution of human hematopoietic function with autologous cryopreserved circulating stem cells. *Exp Hematol* 1986; 14: 192-6.
- Juttner CA, To LB, Haylock DN, Branford A, Kimber RJ. Circulating autologous stem cells collected in very early remission from acute non-lymphoblastic leukaemia produce prompt but incomplete haemopoietic reconstitution after high dose melphalan or supralethal chemoradiotherapy. *Br J Haematol* 1985; 61: 739-45.
- Korbling M, Dorken B, Ho AD, Pezzutto A, Hunstein W, Fliedner TM. Autologous transplantation of blood-derived hemopoietic stem cells after myeloablative therapy in a patient with Burkitt's lymphoma. *Blood* 1986; 67: 529-32.
- Welte K, Platzer E, Lu L, Gabrilove JL, Levi E, Mertelsmann R, *et al.* Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor. *Proc Natl Acad Sci USA* 1985; 82: 1526-30.
- Byrne PV, Heit WF, March CJ. Human granulocyte-macrophage colony-stimulating factor purified from a Hodgkin's tumor cell line. *Biochim Biophys Acta* 1986; 874: 266-73.
- Beyer J, Schwella N, Zingsem J, Strohscheer I, Schwaner I, Oettle H, *et al.* Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. *J Clin Oncol* 1995; 13: 1328-35.
- Hartmann O, Le Coroller AG, Blaise D, Michon J, Philip I, Norolf F, *et al.* Peripheral blood stem cell and bone marrow transplantation for solid tumors and lymphomas: hematologic recovery and costs. A randomized, controlled trial. *Ann Intern Med* 1997; 126: 600-7.
- Schmitz N, Linch DC, Dreger P, Goldstone AH, Boogaerts MA, Ferrant A, *et al.* Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. *Lancet* 1996; 347: 353-7.
- Korbling M, Anderlini P. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? *Blood* 2001; 98: 2900-8.
- Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, *et al.* Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 2001; 344: 175-81.
- Champlin RE, Schmitz N, Horowitz MM, Chappuis B, Chopra R, Cornelissen JJ, *et al.* Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood* 2000; 95: 3702-9.
- Nemunaitis J, Anasetti C, Buckner CD, Appelbaum FR, Shannon-Dorcy K, Hansen J, *et al.* Long-term follow-up of 103 patients who received recombinant human granulocyte-macrophage colony-stimulating factor after unrelated donor bone marrow transplantation. *Blood* 1993; 81: 865.
- Schriber JR, Chao NJ, Long GD, Negrin RS, Tierney DK, Kusnierz-Glaz C, *et al.* Granulocyte colony-stimulating factor after allogeneic bone marrow transplantation. *Blood* 1994; 84: 1680-4.
- Spitzer G, Adkins D, Mathews M, Velasquez W, Bowers C, Dunphy F, *et al.* Randomized comparison of G-CSF + GM-CSF vs G-CSF alone for mobilization of peripheral blood stem cells: effects on hematopoietic recovery after high-dose chemotherapy. *Bone Marrow Transplant* 1997; 20: 921-30.
- Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, *et al.* G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 2002; 3: 687-94.
- Semerad CL, Christopher MJ, Liu F, Short B, Simmons PJ, Winkler I, *et al.* G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. *Blood* 2005; 106: 3020-7.
- Levesque JP, Henty J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during

- hematopoietic stem cell mobilization induced by G-CSF or cyclophosphamide. *J Clin Invest* 2003; 111: 187-96.
- [26] Levesque JP, Hendy J, Takamatsu Y, Williams B, Winkler IG, Simmons PJ. Mobilization by either cyclophosphamide or granulocyte colony-stimulating factor transforms the bone marrow into a highly proteolytic environment. *Exp Hematol* 2002; 30: 440-9.
- [27] Levesque JP, Takamatsu Y, Nilsson SK, Haylock DN, Simmons PJ. Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. *Blood* 2001; 98: 1289-97.
- [28] Winkler IG, Hendy J, Coughlin P, Horvath A, Levesque JP. Serine protease inhibitors serpin1 and serpin3 are down-regulated in bone marrow during hematopoietic progenitor mobilization. *J Exp Med* 2005; 201: 1077-88.
- [29] Levesque JP, Liu F, Simmons PJ, Betsuyaku T, Senior RM, Pham C, *et al.* Characterization of hematopoietic progenitor mobilization in protease-deficient mice. *Blood* 2004; 104: 65-72.
- [30] Pelus LM, Bian H, King AG, Fukuda S. Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. *Blood* 2004; 103: 110-9.
- [31] Puijitt JF, Verzaal P, van Os R, de Kruijff EJ, van Schie ML, Mantovani A, *et al.* Neutrophils are indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice. *Proc Natl Acad Sci USA* 2002; 99: 6228-33.
- [32] Craddock CF, Nakamoto B, Andrews RG, Priestley GV, Papayannopoulou T. Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice. *Blood* 1997; 90: 4779-88.
- [33] Kikuta T, Shimazaki C, Ashihara E, Sudo Y, Hirai H, Sumikuma T, *et al.* Mobilization of hematopoietic primitive and committed progenitor cells into blood in mice by anti-vascular adhesion molecule-1 antibody alone or in combination with granulocyte colony-stimulating factor. *Exp Hematol* 2000; 28: 311-7.
- [34] Papayannopoulou T, Nakamoto B. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci USA* 1993; 90: 9374-8.
- [35] Bullard DC, Kunkel EJ, Kubo H, Hicks MJ, Lorenzo I, Doyle NA, *et al.* Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. *J Exp Med* 1996; 183: 2329-36.
- [36] Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell* 1996; 84: 563-74.
- [37] Pilarski LM, Pruski E, Wizniak J, Paine D, Seeberger K, Mant MJ, *et al.* Potential role for hyaluronan and the hyaluronan receptor RHAMM in mobilization and trafficking of hematopoietic progenitor cells. *Blood* 1999; 93: 2918-27.
- [38] Beguin Y, Baudoux E, Sautois B, Fraipont V, Schaaf-Lafontaine N, Pereira M, *et al.* Hematopoietic recovery in cancer patients after transplantation of autologous peripheral blood CD34+ cells or unmanipulated peripheral blood stem and progenitor cells. *Transfusion* 1998; 38: 199-208.
- [39] Bender JG, To LB, Williams S, Schwartzberg LS. Defining a therapeutic dose of peripheral blood stem cells. *J Hematother* 1992; 1: 329-41.
- [40] Bensinger W, Appelbaum F, Rowley S, Storb R, Sanders J, Lilleby K, *et al.* Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol* 1995; 13: 2547-55.
- [41] Glaspy JA, Shpall EJ, LeMaistre CF, Briddell RA, Menchaca DM, Turner SA, *et al.* Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients. *Blood* 1997; 90: 2939-51.
- [42] Haas R, Moos M, Karcher A, Mohle R, Witt B, Goldschmidt H, *et al.* Sequential high-dose therapy with peripheral-blood progenitor-cell support in low-grade non-Hodgkin's lymphoma. *J Clin Oncol* 1994; 12: 1685-92.
- [43] Passos-Coelho JL, Braine HG, Davis JM, Huelskamp AM, Schepers KG, Ohly K, *et al.* Predictive factors for peripheral-blood progenitor-cell collections using a single large-volume leukapheresis after cyclophosphamide and granulocyte-macrophage colony-stimulating factor mobilization. *J Clin Oncol* 1995; 13: 705-14.
- [44] Reiffers J, Faberes C, Boiron JM, Marit G, Foures C, Ferrer AM, *et al.* Peripheral blood progenitor cell transplantation in 118 patients with hematological malignancies: analysis of factors affecting the rate of engraftment. *J Hematother* 1994; 3: 185-91.
- [45] Weaver CH, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L, *et al.* An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; 86: 3961-9.
- [46] Pecora AL, Preti RA, Gleim GW, Jennis A, Zahos K, Cantwell S, *et al.* CD34+CD33- cells influence days to engraftment and transfusion requirements in autologous blood stem-cell recipients. *J Clin Oncol* 1998; 16: 2093-104.
- [47] Siena S, Schiavo R, Pedrazzoli P, Carlo-Stella C. Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy. *J Clin Oncol* 2000; 18: 1360-77.
- [48] To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. *Blood* 1997; 89: 2233-58.
- [49] Devine SM, Brown RA, Mathews V, Trinkaus K, Khoury H, Adkins D *et al.* Reduced risk of acute GVHD following mobilization of HLA-identical sibling donors with GM-CSF alone. *Bone Marrow Transplant* 2005; 36: 531-8.
- [50] Gazitt Y. Comparison between granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the mobilization of peripheral blood stem cells. *Curr Opin Hematol* 2002; 9: 190-8.
- [51] Lane TA, Law P, Maruyama M, Young D, Burgess J, Mullen M, *et al.* Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF: potential role in allogeneic marrow transplantation. *Blood* 1995; 85: 275-82.
- [52] Peters WP, Rosner G, Ross M, Vredenburg J, Meisenberg B, Gilbert C, *et al.* Comparative effects of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on priming peripheral blood progenitor cells for use with autologous bone marrow after high-dose chemotherapy. *Blood* 1993; 81: 1709-19.
- [53] Weaver CH, Schulman KA, Wilson-Relyea B, Birch R, West W, Buckner CD. Randomized trial of filgrastim, sargramostim, or sequential sargramostim and filgrastim after myelosuppressive chemotherapy for the harvesting of peripheral-blood stem cells. *J Clin Oncol* 2000; 18: 43-53.
- [54] Bishop MR, Anderson JR, Jackson JD, Bierman PJ, Reed EC, Vose JM, *et al.* High-dose therapy and peripheral blood progenitor cell transplantation: effects of recombinant human granulocyte-macrophage colony-stimulating factor on the autograft. *Blood* 1994; 83: 610-6.
- [55] Demirel T, Ayli M, Ozcan M, Gunel N, Haznedar R, Dagli M, *et al.* Mobilization of peripheral blood stem cells with chemotherapy and recombinant human granulocyte colony-stimulating factor (rhG-CSF): a randomized evaluation of different doses of rhG-CSF. *Br J Haematol* 2002; 116: 468-74.
- [56] Engelhardt M, Bertz H, Afting M, Waller CF, Finke J. High-versus standard-dose filgrastim (rhG-CSF) for mobilization of peripheral-blood progenitor cells from allogeneic donors and CD34(+) immunoselection. *J Clin Oncol* 1999; 17: 2160-72.
- [57] Nademanee A, Sniecinski I, Schmidt GM, Dagens AC, O'Donnell MR, Snyder DS, *et al.* High-dose therapy followed by autologous peripheral-blood stem-cell transplantation for patients with Hodgkin's disease and non-Hodgkin's lymphoma using unprimed and granulocyte colony-stimulating factor-mobilized peripheral-blood stem cells. *J Clin Oncol* 1994; 12: 2176-86.
- [58] Kim S, Kim HJ, Park JS, Lee J, Chi HS, Park CJ, *et al.* Prospective randomized comparative observation of single- vs split-dose lenograstim to mobilize peripheral blood progenitor cells following chemotherapy in patients with multiple myeloma or non-Hodgkin's lymphoma. *Ann Hematol* 2005; 84: 742-7.
- [59] Komeno Y, Kanda Y, Hamaki T, Mitani K, Iijima K, Ueyama J, *et al.* A randomized controlled trial to compare once- versus twice-daily filgrastim for mobilization of peripheral blood stem cells

- from healthy donors. *Biol Blood Marrow Transplant* 2006; 12: 408-13.
- [60] Kroger N, Renges H, Kruger W, Gutensohn K, Loliger C, Carrero I, *et al.* A randomized comparison of once versus twice daily recombinant human granulocyte colony-stimulating factor (filgrastim) for stem cell mobilization in healthy donors for allogeneic transplantation. *Br J Haematol* 2000; 111: 761-5.
- [61] Henon PR, Liang H, Beck-Wirth G, Eisenmann JC, Lepers M, Wunder E, *et al.* Comparison of hematopoietic and immune recovery after autologous bone marrow or blood stem cell transplants. *Bone Marrow Transplant* 1992; 9: 285-91.
- [62] Narayanasami U, Kanteti R, Morelli J, Klekar A, Al-Olama A, Keating C, *et al.* Randomized trial of filgrastim versus chemotherapy and filgrastim mobilization of hematopoietic progenitor cells for rescue in autologous transplantation. *Blood* 2001; 98: 2059-64.
- [63] Desikan KR, Barlogie B, Jagannath S, Vesole DH, Siegel D, Fassas A, *et al.* Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol* 1998; 16: 1547-53.
- [64] Dingli D, Nowakowski GS, Dispenzieri A, Lacy MQ, Hayman S, Litzow MR, *et al.* Cyclophosphamide mobilization does not improve outcome in patients receiving stem cell transplantation for multiple myeloma. *Clin Lymphoma Myeloma* 2006; 6: 384-8.
- [65] Lee JL, Kim S, Kim SW, Kim EK, Kim SB, Kang YK, *et al.* ESHAP plus G-CSF as an effective peripheral blood progenitor cell mobilization regimen in pretreated non-Hodgkin's lymphoma: comparison with high-dose cyclophosphamide plus G-CSF. *Bone Marrow Transplant* 2005; 35: 449-54.
- [66] Krishnan A, Bhatia S, Slovak ML, Arber DA, Niland JC, Nadeemane A, *et al.* Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. *Blood* 2000; 95: 1588-93.
- [67] Olavarria E, Kanfer EJ. Selection and use of chemotherapy with hematopoietic growth factors for mobilization of peripheral blood progenitor cells. *Curr Opin Hematol* 2000; 7: 191-6.
- [68] Tarella C, Zallio F, Caracciolo D, Cherasco C, Bondesan P, Gavarotti P, *et al.* Hemopoietic progenitor cell mobilization and harvest following an intensive chemotherapy debulking in indolent lymphoma patients. *Stem Cells* 1999; 17: 55-61.
- [69] Bourhis JH, Bouko Y, Koscielny S, Bakkus M, Greinix H, Derigs G, *et al.* Relapse risk after autologous transplantation in patients with newly diagnosed myeloma is not related with infused tumor cell load and the outcome is not improved by CD34+ cell selection: long term follow-up of an EBMT phase III randomized study. *Haematologica* 2007; 92: 1083-90.
- [70] Morris CL, Siegel E, Barlogie B, Cottler-Fox M, Lin P, Fassas A, *et al.* Mobilization of CD34+ cells in elderly patients ( $\geq 70$  years) with multiple myeloma: influence of age, prior therapy, platelet count and mobilization regimen. *Br J Haematol* 2003; 120: 413-23.
- [71] Dreger P, Kloss M, Petersen B, Haferlach T, Loffler H, Loeffler M, *et al.* Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts. *Blood* 1995; 86: 3970-8.
- [72] Moskowitz CH, Glassman JR, Wuest D, Maslak P, Reich L, Gucciardo A, *et al.* Factors affecting mobilization of peripheral blood progenitor cells in patients with lymphoma. *Clin Cancer Res* 1998; 4: 311-6.
- [73] Kobbe G, Sohngen D, Bauser U, Schneider P, Germing U, Thiele KP, *et al.* Factors influencing G-CSF-mediated mobilization of hematopoietic progenitor cells during steady-state hematopoiesis in patients with malignant lymphoma and multiple myeloma. *Ann Hematol* 1999; 78: 456-62.
- [74] Pery AR, Watts MJ, Peniket AJ, Goldstone AH, Linch DC. Progenitor cell yields are frequently poor in patients with histologically indolent lymphomas especially when mobilized within 6 months of previous chemotherapy. *Bone Marrow Transplant* 1998; 21: 1201-5.
- [75] Gazitt Y, Liu Q. Plasma levels of SDF-1 and expression of SDF-1 receptor on CD34+ cells in mobilized peripheral blood of non-Hodgkin's lymphoma patients. *Stem Cells* 2001; 19: 37-45.
- [76] Anderlini P, Przepiorka D, Seong D, Miller P, Sundberg J, Lichtiger B, *et al.* Clinical toxicity and laboratory effects of granulocyte colony-stimulating factor (filgrastim) mobilization and blood stem cell apheresis from normal donors, and analysis of charges for the procedures. *Transfusion* 1996; 36: 590-5.
- [77] Anderlini P, Przepiorka D, Korbling M, Champlin R. Blood stem cell procurement: donor safety issues. *Bone Marrow Transplant* 1998; 21(Suppl 3): S35-9.
- [78] Fortanier C, Kuentz M, Sutton L, Milpied N, Michalet M, Macquart-Moulin G, *et al.* Healthy sibling donor anxiety and pain during bone marrow or peripheral blood stem cell harvesting for allogeneic transplantation: results of a randomised study. *Bone Marrow Transplant* 2002; 29: 145-9.
- [79] Murata M, Harada M, Kato S, Takahashi S, Ogawa H, Okamoto S, *et al.* Peripheral blood stem cell mobilization and apheresis: analysis of adverse events in 94 normal donors. *Bone Marrow Transplant* 1999; 24: 1065-71.
- [80] Rowley SD, Donaldson G, Lilleby K, Bensinger WI, Appelbaum FR. Experiences of donors enrolled in a randomized study of allogeneic bone marrow or peripheral blood stem cell transplantation. *Blood* 2001; 97: 2541-8.
- [81] Balaguer H, Galmes A, Ventayol G, Bargay J, Besalduch J. Splenic rupture after granulocyte-colony-stimulating factor mobilization in a peripheral blood progenitor cell donor. *Transfusion* 2004; 44: 1260-1.
- [82] Falzetti F, Aversa F, Minelli O, Tabilio A. Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor. *Lancet* 1999; 353: 555.
- [83] Becker PS, Wagle M, Matous S, Swanson RS, Pihan G, Lowry PA, *et al.* Spontaneous splenic rupture following administration of granulocyte colony-stimulating factor (G-CSF): occurrence in an allogeneic donor of peripheral blood stem cells. *Biol Blood Marrow Transplant* 1997; 3: 45-9.
- [84] Dincer AP, Gottschall J, Margolis DA. Splenic rupture in a parental donor undergoing peripheral blood progenitor cell mobilization. *J Pediatr Hematol Oncol* 2004; 26: 761-3.
- [85] Kroger N, Renges H, Sonnenberg S, Kruger W, Gutensohn K, Dielschneider T, *et al.* Stem cell mobilisation with 16 microg/kg vs 10 microg/kg of G-CSF for allogeneic transplantation in healthy donors. *Bone Marrow Transplant* 2002; 29: 727-30.
- [86] Adler BK, Salzman DE, Carabasi MH, Vaughan WP, Reddy VV, Prchal JT. Fatal sickle cell crisis after granulocyte colony-stimulating factor administration. *Blood* 2001; 97: 3313-4.
- [87] Horowitz MM, Confer DL. Evaluation of hematopoietic stem cell donors. *Hematology Am Soc Hematol Educ Program* 2005: 469-75.
- [88] Kang EM, Areman EM, David-Ocampo V, Fitzhugh C, Link ME, Read EJ, *et al.* Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. *Blood* 2002; 99: 850-5.
- [89] de Vries EG, Willemsse PH, Biesma B, Stern AC, Limburg PC, Vellenga E. Flare-up of rheumatoid arthritis during GM-CSF treatment after chemotherapy. *Lancet* 1991; 338: 517-8.
- [90] Fukumoto Y, Miyamoto T, Okamura T, Gondo H, Iwasaki H, Horiuchi T, *et al.* Angina pectoris occurring during granulocyte colony-stimulating factor-combined preparatory regimen for autologous peripheral blood stem cell transplantation in a patient with acute myelogenous leukaemia. *Br J Haematol* 1997; 97: 666-8.
- [91] Hill JM, Syed MA, Arai AE, Powell TM, Paul JD, Zalos G, *et al.* Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J Am Coll Cardiol* 2005; 46: 1643-8.
- [92] Lindemann A, Rumberger B. Vascular complications in patients treated with granulocyte colony-stimulating factor (G-CSF). *Eur J Cancer* 1993; 29A: 2338-9.
- [93] Gutierrez-Delgado F, Bensinger W. Safety of granulocyte colony-stimulating factor in normal donors. *Curr Opin Hematol* 2001; 8: 155-60.
- [94] Couban S, Simpson DR, Barnett MJ, Bredeson C, Hubsch L, Howson-Jan K, *et al.* A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* 2002; 100: 1525-31.
- [95] Cutler C, Giri S, Jayapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft-versus-host disease after allogeneic pe-

- ripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol* 2001; 19: 3685-91.
- [96] Mohty M, Kuentz M, Michallet M, Bourhis JH, Milpied N, Sutton L, *et al.* Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood* 2002; 100: 3128-34.
- [97] Brown RA, Adkins D, Goodnough LT, Haug JS, Todd G, Wehde M, *et al.* Factors that influence the collection and engraftment of allogeneic peripheral-blood stem cells in patients with hematologic malignancies. *J Clin Oncol* 1997; 15: 3067-74.
- [98] Bittencourt H, Rocha V, Chevret S, Socie G, Esperou H, Devergie A, *et al.* Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002; 99: 2726-33.
- [99] Davies SM, Kollman C, Anasetti C, Antin JH, Gajewski J, Casper JT, *et al.* Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. *Blood* 2000; 96: 4096-102.
- [100] Mavroudis D, Read E, Cottler-Fox M, Couriel D, Mollidrem J, Carter C, *et al.* CD34+ cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies. *Blood* 1996; 88: 3223-9.
- [101] Przepiorka D, Smith TL, Folloder J, Khouri I, Ueno NT, Mehra R, *et al.* Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 1999; 94: 1465-70.
- [102] Baron F, Maris MB, Storer BE, Sandmaier BM, Panse JP, Chauncey TR, *et al.* High doses of transplanted CD34+ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. *Leukemia* 2005; 19: 822-8.
- [103] Zaucha JM, Gooley T, Bensinger WI, Heimfeld S, Chauncey TR, Zaucha R, *et al.* CD34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transplantation. *Blood* 2001; 98: 3221-7.
- [104] Mohty M, Bagattini S, Chabannon C, Faucher C, Bardou VJ, Bilger K, *et al.* CD8+ T cell dose affects development of acute graft-versus-host disease following reduced-intensity conditioning allogeneic peripheral blood stem cell transplantation. *Exp Hematol* 2004; 32: 1097-102.
- [105] Heimfeld S. HLA-identical stem cell transplantation: is there an optimal CD34 cell dose? *Bone Marrow Transplant* 2003; 31: 839-45.
- [106] Heimfeld S. Bone marrow transplantation: how important is CD34 cell dose in HLA-identical stem cell transplantation? *Leukemia* 2003; 17: 856-8.
- [107] Panse JP, Heimfeld S, Guthrie KA, Maris MB, Maloney DG, Baril BB, *et al.* Allogeneic peripheral blood stem cell graft composition affects early T-cell chimerism and later clinical outcomes after non-myeloablative conditioning. *Br J Haematol* 2005; 128: 659-67.
- [108] Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 2000; 95: 2484-90.
- [109] Pan L, Delmonte J Jr, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. *Blood* 1995; 86: 4422-9.
- [110] Liu YJ, Blom B. Introduction: TH2-inducing DC2 for immunotherapy. *Blood* 2000; 95: 2482-3.
- [111] Reddy V. Granulocyte colony-stimulating factor mobilization alters dendritic cell cytokine production and initiates T helper 2 polarization prior to host alloantigen presentation. *Blood* 2000; 96: 2635.
- [112] Fischmeister G, Kurz M, Haas OA, Micksche M, Buchinger P, Printz D, *et al.* G-CSF versus GM-CSF for stimulation of peripheral blood progenitor cells (PBPC) and leukocytes in healthy volunteers: comparison of efficacy and tolerability. *Ann Hematol* 1999; 78: 117-23.
- [113] Sohn SK, Kim JG, Seo KW, Chae YS, Jung JT, Suh JS, *et al.* GM-CSF-based mobilization effect in normal healthy donors for allogeneic peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2002; 30: 81-6.
- [114] Edinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S, *et al.* CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* 2003; 9: 1144-50.
- [115] Parajuli P, Mosley RL, Pisarev V, Chavez J, Ulrich A, Varney M, *et al.* Flt3 ligand and granulocyte-macrophage colony-stimulating factor preferentially expand and stimulate different dendritic and T-cell subsets. *Exp Hematol* 2001; 29: 1185-93.
- [116] Taylor PA, Lees CJ, Blazar BR. The infusion of *ex vivo* activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 2002; 99: 3493-9.
- [117] Vasu C, Dogan RN, Holterman MJ, Prabhakar BS. Selective induction of dendritic cells using granulocyte macrophage-colony stimulating factor, but not fms-like tyrosine kinase receptor 3-ligand, activates thyroglobulin-specific CD4+/CD25+ T cells and suppresses experimental autoimmune thyroiditis. *J Immunol* 2003; 170: 5511-22.
- [118] Anderlini P, Rizzo JD, Nugent ML, Schmitz N, Champlin RE, Horowitz MM. Peripheral blood stem cell donation: an analysis from the International Bone Marrow Transplant Registry (IBMTR) and European Group for Blood and Marrow Transplant (EBMT) databases. *Bone Marrow Transplant* 2001; 27: 689-92.
- [119] Anderlini P, Donato M, Chan KW, Huh YO, Gee AP, Lauppe MJ, *et al.* Allogeneic blood progenitor cell collection in normal donors after mobilization with filgrastim: the M.D. Anderson Cancer Center experience. *Transfusion* 1999; 39: 555-60.
- [120] Majolino I, Cavallaro AM, Bacigalupo A, Rambaldi A, Falda M, Locatelli F, *et al.* Mobilization and collection of PBSC in healthy donors: a retrospective analysis of the Italian Bone Marrow Transplantation Group (GITMO). *Haematologica* 1997; 82: 47-52.
- [121] de la Rubia J, Arbona C, de Arriba F, del Canizo C, Brunet S, Zamora C, *et al.* Analysis of factors associated with low peripheral blood progenitor cell collection in normal donors. *Transfusion* 2002; 42: 4-9.
- [122] Lysak D, Koza V, Jindra P. Factors affecting PBSC mobilization and collection in healthy donors. *Transfus Apher Sci* 2005; 33: 275-83.
- [123] Suzuya H, Watanabe T, Nakagawa R, Watanabe H, Okamoto Y, Onishi T, *et al.* Factors associated with granulocyte colony-stimulating factor-induced peripheral blood stem cell yield in healthy donors. *Vox Sang* 2005; 89: 229-35.
- [124] Burt RK, Fassas A, Snowden J, van Laar JM, Kozak T, Wulffraat NM, *et al.* Collection of hematopoietic stem cells from patients with autoimmune diseases. *Bone Marrow Transplant* 2001; 28: 1-12.
- [125] Gottenberg JE, Roux S, Desmoulin F, Clerc D, Mariette X. Granulocyte colony-stimulating factor therapy resulting in a flare of systemic lupus erythematosus: comment on the article by Yang and Hamilton. *Arthritis Rheum* 2001; 44: 2458-60.
- [126] Stricker RB, Goldberg B. G-CSF and exacerbation of rheumatoid arthritis. *Am J Med* 1996; 100: 665-6.
- [127] Hernandez JM, Castilla C, Gutierrez NC, Isidro IM, Delgado M, de las Rivas J, *et al.* Mobilisation with G-CSF in healthy donors promotes a high but temporal deregulation of genes. *Leukemia* 2005; 19: 1088-91.
- [128] Nagler A, Korenstein-Ilan A, Amiel A, Avivi L. Granulocyte colony-stimulating factor generates epigenetic and genetic alterations in lymphocytes of normal volunteer donors of stem cells. *Exp Hematol* 2004; 32: 122-30.
- [129] Nemunaitis J, Rabinowe SN, Singer JW, Bierman PJ, Vose JM, Freedman AS, *et al.* Recombinant granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for lymphoid cancer. *N Engl J Med* 1991; 324: 1773-8.
- [130] Rabinowe SN, Nemunaitis J, Armitage J, Nadler LM. The impact of myeloid growth factors on engraftment following autologous bone marrow transplantation for malignant lymphoma. *Semin Hematol* 1991; 28: 6-16.
- [131] Rabinowe SN, Neuberger D, Bierman PJ, Vose JM, Nemunaitis J, Singer JW, *et al.* Long-term follow-up of a phase III study of recombinant human granulocyte-macrophage colony-stimulating factor after autologous bone marrow transplantation for lymphoid malignancies. *Blood* 1993; 81: 1903-8.

- [132] Pavletic ZS, Bishop MR, Tarantolo SR, Martin-Algarra S, Bierman PJ, Vose JM, *et al.* Hematopoietic recovery after allogeneic blood stem-cell transplantation compared with bone marrow transplantation in patients with hematologic malignancies. *J Clin Oncol* 1997; 15: 1608-16.
- [133] Fowler DH. Shared biology of GVHD and GVT effects: potential methods of separation. *Crit Rev Oncol Hematol* 2006; 57: 225-44.
- [134] Sloand EM, Kim S, Maciejewski JP, Van Rhee F, Chaudhuri A, Barrett J, *et al.* Pharmacologic doses of granulocyte colony-stimulating factor affect cytokine production by lymphocytes *in vitro* and *in vivo*. *Blood* 2000; 95: 2269-74.
- [135] Volpi I, Perruccio K, Tosti A, Capanni M, Ruggeri L, Posati S, *et al.* Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood* 2001; 97: 2514-21.
- [136] Dekker A, Bulley S, Beyene J, Dupuis LL, Doyle JJ, Sung L. Meta-analysis of randomized controlled trials of prophylactic granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor after autologous and allogeneic stem cell transplantation. *J Clin Oncol* 2006; 24: 5207-15.
- [137] Bohlius J, Reiser M, Schwarzer G, Engert A. Impact of granulocyte colony-stimulating factor (CSF) and granulocyte-macrophage CSF in patients with malignant lymphoma: a systematic review. *Br J Haematol* 2003; 122: 413-23.
- [138] Bohlius J, Reiser M, Schwarzer G, Engert A. Granulopoiesis-stimulating factors to prevent adverse effects in the treatment of malignant lymphoma. *Cochrane Database Syst Rev* 2004; CD003189.
- [139] Lyman GH, Kuderer NM, Djulbegovic B. Prophylactic granulocyte colony-stimulating factor in patients receiving dose-intensive cancer chemotherapy: a meta-analysis. *Am J Med* 2002; 112: 406-11.
- [140] Ho VT, Mirza NQ, Junco Dd D, Okamura T, Przepiorka D. The effect of hematopoietic growth factors on the risk of graft-vs-host disease after allogeneic hematopoietic stem cell transplantation: a meta-analysis. *Bone Marrow Transplant* 2003; 32: 771-5.
- [141] Bishop MR, Tarantolo SR, Geller RB, Lynch JC, Bierman PJ, Pavletic ZS, *et al.* A randomized, double-blind trial of filgrastim (granulocyte colony-stimulating factor) versus placebo following allogeneic blood stem cell transplantation. *Blood* 2000; 96: 80-5.
- [142] Khoury HJ, Loberiza FR, Jr., Ringden O, Barrett AJ, Bolwell BJ, Cahn JY, *et al.* Impact of posttransplantation G-CSF on outcomes of allogeneic hematopoietic stem cell transplantation. *Blood* 2006; 107: 1712-6.
- [143] Eapen M, Horowitz MM, Klein JP, Champlin RE, Loberiza FR Jr, Ringden O, *et al.* Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: the Histocompatibility and Alternate Stem Cell Source Working Committee of the International Bone Marrow Transplant Registry. *J Clin Oncol* 2004; 22: 4872-80.
- [144] Remberger M, Naseh N, Aschan J, Barkholt L, LeBlanc K, Svenberg P, *et al.* G-CSF given after haematopoietic stem cell transplantation using HLA-identical sibling donors is associated to a higher incidence of acute GVHD II-IV. *Bone Marrow Transplant* 2003; 32: 217-23.
- [145] Ringden O, Labopin M, Gorin NC, Le Blanc K, Rocha V, Gluckman E, *et al.* Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemia increases the risk of graft-versus-host disease and death: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol* 2004; 22: 416-23.
- [146] Bernstein SH, Nademanee AP, Vose JM, Tricot G, Fay JW, Negrin RS, *et al.* A multicenter study of platelet recovery and utilization in patients after myeloablative therapy and hematopoietic stem cell transplantation. *Blood* 1998; 91: 3509-17.
- [147] Ener RA, Meglathery SB, Cuhaci B, Topolsky D, Styler MJ, Crilley P, *et al.* Use of granulocyte colony-stimulating factor after high-dose chemotherapy and autologous peripheral blood stem cell transplantation: what is the optimal timing? *Am J Clin Oncol* 2001; 24: 19-25.
- [148] Faucher C, Le Corroller AG, Chabannon C, Novakovitch G, Manonni P, Moatti JP, *et al.* Administration of G-CSF can be delayed after transplantation of autologous G-CSF-primed blood stem cells: a randomized study. *Bone Marrow Transplant* 1996; 17: 533-6.
- [149] Vey N, Molnar S, Faucher C, Le Corroller AG, Stoppa AM, Viens P, *et al.* Delayed administration of granulocyte colony-stimulating factor after autologous bone marrow transplantation: effect on granulocyte recovery. *Bone Marrow Transplant* 1994; 14: 779-82.
- [150] Ciernik IF, Schanz U, Gmur J. Delaying treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for hematological malignancies: a prospective randomized trial. *Bone Marrow Transplant* 1999; 24: 147-51.
- [151] Ojeda E, Garcia-Bustos J, Agaudo MJ, Quevedo E, Arrieta R, Jimenez V, *et al.* Is filgrastim as useless after peripheral blood stem cell transplantation for adults as it could be for children? *Blood* 1999; 93: 3565-6.
- [152] Bolwell B, Goormastic M, Dannley R, Andresen S, Overmoyer B, Mendez Z, *et al.* G-CSF post-autologous progenitor cell transplantation: a randomized study of 5, 10, and 16 micrograms/kg/day. *Bone Marrow Transplant* 1997; 19: 215-9.
- [153] Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; 11: 945-56.
- [154] De Clercq E. The bicyclam AMD3100 story. *Nat Rev Drug Discov* 2003; 2: 581-7.
- [155] Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, *et al.* Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother* 2000; 44: 1667-73.
- [156] Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, *et al.* Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003; 102: 2728-30.
- [157] Lack NA, Green B, Dale DC, Calandra GB, Lee H, MacFarland RT, *et al.* A pharmacokinetic-pharmacodynamic model for the mobilization of CD34+ hematopoietic progenitor cells by AMD3100. *Clin Pharmacol Ther* 2005; 77: 427-36.
- [158] Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, *et al.* 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* 2005; 45: 295-300.
- [159] Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, *et al.* Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol* 2004; 22: 1095-102.
- [160] Flomenberg N, Devine SM, DiPersio JF, Liesveld JL, McCarty JM, Rowley SD, *et al.* The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005; 106: 1867-74.
- [161] Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, *et al.* Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 2005; 201: 1307-18.
- [162] Gazitt Y. Homing and mobilization of hematopoietic stem cells and hematopoietic cancer cells are mirror image processes, utilizing similar signaling pathways and occurring concurrently: circulating cancer cells constitute an ideal target for concurrent treatment with chemotherapy and antineoplastic-specific antibodies. *Leukemia* 2004; 18: 1-10.
- [163] Dawson MA, Schwarzer AP, Muirhead JL, Bailey MJ, Bollard GM, Spencer A. Successful mobilization of peripheral blood stem cells using recombinant human stem cell factor in heavily pretreated patients who have failed a previous attempt with a granulocyte colony-stimulating factor-based regimen. *Bone Marrow Transplant* 2005; 36: 389-96.
- [164] Morstyn G, Brown S, Gordon M, Crawford J, Demetri G, Rich W, *et al.* Stem cell factor is a potent synergistic factor in hematopoiesis. *Oncology* 1994; 51: 205-14.
- [165] Moskowitz CH, Stiff P, Gordon MS, McNiece I, Ho AD, Costa JJ, *et al.* Recombinant methionyl human stem cell factor and filgrastim for peripheral blood progenitor cell mobilization and transplantation in non-Hodgkin's lymphoma patients--results of a phase I/II trial. *Blood* 1997; 89: 3136-47.

- [166] Costa JJ, Demetri GD, Harrist TJ, Dvorak AM, Hayes DF, Merica EA, *et al.* Recombinant human stem cell factor (kit ligand) promotes human mast cell and melanocyte hyperplasia and functional activation *in vivo*. *J Exp Med* 1996; 183: 2681-6.
- [167] Hepburn TW, Hart TK, Horton VL, Sellers TS, Tobia LP, Urbanski JJ, *et al.* Pharmacokinetics and tissue distribution of SB-251353, a novel human CXC chemokine, after intravenous administration to mice. *J Pharmacol Exp Ther* 2001; 298: 886-93.
- [168] King AG, Horowitz D, Dillon SB, Levin R, Farese AM, MacVittie TJ, *et al.* Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta. *Blood* 2001; 97: 1534-42.
- [169] Pelus LM, Horowitz D, Cooper SC, King AG. Peripheral blood stem cell mobilization. A role for CXC chemokines. *Crit Rev Oncol Hematol* 2002; 43: 257-75.
- [170] Johnston E, Crawford J, Blackwell S, Bjurstrom T, Lockbaum P, Roskos L, *et al.* Randomized, dose-escalation study of SD/01 compared with daily filgrastim in patients receiving chemotherapy. *J Clin Oncol* 2000; 18: 2522-8.
- [171] Molineux G, Kinstler O, Briddell B, Hartley C, McElroy P, Kerzic P, *et al.* A new form of Filgrastim with sustained duration *in vivo* and enhanced ability to mobilize PBPC in both mice and humans. *Exp Hematol* 1999; 27: 1724-34.
- [172] Bruns I, Steidl U, Scheid C, Hübel K, Fenk R, Neumann F, *et al.* Transplantation of peripheral blood stem cells mobilized by chemotherapy and single dose pegylated G-CSF in patients with multiple myeloma: Equivalence of 6 mg and 12 mg pegfilgrastim. *Blood* 2004; 104.
- [173] Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006; 441: 1075-9.
- [174] Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood* 2005; 105: 2631-9.
- [175] Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003; 425: 841-6.