Optimization of Peripheral Blood Stem Cell Mobilization

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Concise Review

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Taner Demirer, C. Dean Buckner, William I. Bensinger
Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Key Words. Peripheral blood stem cell • Stem cell collections • Hematopoietic growth factors • Chemotherapy

Abstract. Peripheral blood stem cells (PBSC) are increasingly utilized in lieu of marrow for hematopoietic support due to the ease of collection and the rapid kinetics of recovery relative to bone marrow (BM). Neutrophil and platelet recovery times after PBSC transplantation average less than 8-12 days after infusion in contrast to the usual two to four weeks experienced after BM transplantation. This has simplified autologous transplantation and made it safer because patients require fewer days of antibiotic and blood component support and are discharged earlier from the hospital. The administration of hematopoietic growth factors during recovery from high-dose chemotherapy increases the number of circulating hematopoietic progenitor cells to levels as much as 1,000-fold greater than levels normally found in blood and 10-50 times greater than with chemotherapy alone. More recently, it has been shown that adequate numbers of PBSC can be collected using growth factors alone without prior chemotherapy. Although not yet universally accepted, the $CD34^+$ cell content of PBSC appears to be the single most powerful predictor of recovery kinetics in patients receiving myeloablative therapy and PBSC infusion. Infusion of $>5 \times 10^6$ $CD34^+$ cells/kg is associated with a rapid engraftment of neutrophils and platelets, although successful engraftment has also been reported with the infusion of $2.5\text{-}5 \times 10^6$ $CD34^+$ cells/kg. By measuring the CD34 or colony forming units-granulocyte-macrophage (CFU-GM) content of PBSC collections, mobilization chemotherapy and cytokine regimens, age, marrow disease, prior radiation and prior chemotherapy treatment have been found to be important factors influencing the numbers of stem cells collected. The current challenge for clinical investigators is to improve methods of identifying patients who will fail to mobilize sufficient numbers of PBSC prior to collection and to utilize new strategies for stem cell mobilization. The relative ease of collection and the rapid engraftment after myeloablative therapy suggest that PBSC will likely supplant marrow for both allogeneic and autologous transplantation in the next five years. *Stem Cells 1996;14:106-116*

Correspondence: Dr. Taner Demirer, The Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104, USA.

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Introduction

Peripheral blood stem cell (PBSC) infusions are being utilized with increasing frequency as a source of marrow repopulating cells following the administration of myeloablative chemoradiotherapy for the treatment of hematologic or other malignancies. PBSC have several advantages when compared to marrow grafts—their procurement does not require hospital admission or exposure to general anesthesia. Furthermore, PBSC have shortened the period of cytopenia following myeloablative therapy compared to marrow. The recovery of both neutrophils and platelets is so much more rapid with growth factor mobilized PBSC than with marrow followed by growth factors that widespread use of PBSC has occurred without randomized comparison studies. In this article, we will review issues concerning the optimization of PBSC collected after hematopoietic growth factor administration.

What Constitutes an Adequate PBSC Collection?

In order to accurately determine the best way to mobilize and collect PBSC, it is important to know what exactly is meant by an adequate collection. The criteria currently used by transplant groups to define an adequate PBSC infusion vary considerably. A number of parameters,

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including nucleated and mononuclear cell number, quantity of colony forming cells-granulocyte-macrophage (CFU-GM) and the number of $CD34^+$ cells (CD34 is an antigen expressed on stem and progenitor cells) present in the PBSC collection have been used to assess the quality of a graft prior to its infusion [1-9]. Currently, there is no standard stem cell assay which is universally accepted. The ideal assay should be rapid, relatively inexpensive and useful for predicting both early and sustained hematologic recovery of both neutrophils and platelets. Because both the CFU-GM and CD34 assays require significant laboratory skill, some transplant centers use total mononucleated cell (MNC) count and attempt to collect at least 4 to 6×10^8 cells/kg. Our own studies and those of others found that the MNC count correlates poorly with engraftment kinetics [1, 3-5]. *Fernandez et al.* reported that neither the number of MNC/kg infused nor the number of CFU-GM/kg infused correlated with time to safe levels of granulocytes or self-sustaining levels of platelets, but did note a correlation between $CD34^+$ cells infused and the speed of hematologic recovery [6]. In other studies, both $CD34^+$ cell dose and CFU-GM graft content have been shown to predict the time to engraftment [5, 10, 11]. *Bender et al.* have reported that rapid engraftment occurs when PBSC infusion contains 20×10^4 CFU-GM/kg or 2×10^6 $CD34^+$ cells/kg [5]. *Schwartzberg* observed in 52 patients receiving chemotherapy-mobilized PBSC that a threshold dose of 2.5×10^6 $CD34^+$ cells/kg and 20×10^4 CFU-GM/kg correlated with rapid engraftment of neutrophils and platelets [11]. In another study which included 243 patients from our institution, patients receiving $>2.5 \times 10^6$ $CD34^+$ cells/kg had more rapid neutrophil ($p = 0.001$) and platelet recovery ($p = 0.0001$) than patients who received $<2.5 \times 10^6$ $CD34^+$ cells/kg. In patients receiving 2.5-5 or $>5 \times 10^6$ $CD34^+$ cells/kg, there was no discernable difference in neutrophil kinetics but there was more rapid recovery of platelets ($p = 0.01$) in patients receiving the higher cell doses [4]. However, the threshold of 5×10^6 $CD34^+$ cells/kg is not absolute, and about 50% of patients who receive $2.5\text{-}5 \times 10^6$ $CD34^+$ cells/kg will still have rapid recovery. *Tricot* reported on a series of 225 patients with myeloma who received PBSC mobilized by cyclophosphamide (CY) and granulocyte-macrophage colony-stimulating factor (GM-CSF) and used alone or with marrow. A threshold dose of 2.5×10^6 $CD34^+$ cells/kg was found for patients receiving <6 months of melphalan, but $>5 \times 10^6$ $CD34^+$ cells/kg were required for rapid platelet recovery in patients who received >12 months of melphalan [12]. This threshold effect for PBSC has also been described using CFU-GM [5, 11].

Rybka et al., in 94 patients, found that the $CD34^+$ cell content of the graft serves as a reliable indicator of engraftment for granulocyte and platelets [7]. In that study, $CD34^+$ cell content correlated significantly with the CFU-GM, burst-forming units-erythroid (BFU-E) and colony forming units-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM) content ($p < 0.0005$), and stepwise linear regression showed significant correlations between $CD34^+$ cell content and days to granulocyte and platelet recovery ($p = 0.004$ and 0.03, respectively) [7]. *Schiller* determined that threshold dose to achieve hematopoietic recovery using PBSC was 2×10^6 $CD34^+$ cells/kg, below which engraftment was prolonged and incomplete [13]. *Weaver et al.* found that $CD34^+$ cell content of PBSC $>5 \times 10^6$ $CD34^+$ cells/kg was associated with an approximate 95% probability of achieving neutrophil and platelet recovery by 21 days after high dose chemotherapy [9]. *Haas et al.* reported a successful hematologic recovery within two weeks in patients receiving $CD34^+$ cells of $>2.5 \times 10^6$/kg [14]. The minimum quantity of PBSC required for successful engraftment is unknown but is probably between $2\text{-}3 \times 10^6$ $CD34^+$ cells/kg. Although there may be some patients who have relatively rapid engraftment below this level, very prolonged platelet recovery has been observed in some patients who received $<2 \times 10^6$ $CD34^+$ cells/kg [3]. At low $CD34^+$ cell doses there is probably little if any advantage of PBSC over marrow due to the slow engraftment tempo.

A few studies have not found $CD34^+$ cell enumeration to be useful for predicting engraftment [15, 16]. In general, those studies had one or more problems including relatively few patients, mobilization by heterogenous methods, marrow infused with PBSC, patients given submyeloablative regimens and the use of post-transplant growth factors, all of which made analyses difficult. The commonly used CFU-GM assay is probably not the best available tool for stem cell enumeration. It clearly

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measures committed cells of granulocyte lineage and, at best, is only a surrogate marker for megakaryocytic precursors, which is why correlations of CFU-GM dose with platelet recovery are less reliable than with neutrophil recovery [1, 2]. Other problems such as interlaboratory variation, significant lag-time between the start of the assay and its read out, and lack of standardization limit the usefulness of in vitro culture techniques for enumerating PBSC content.

Factors Correlating with Mobilization Yield

Several reports have indicated that there is a significant interpatient variation in the ability to mobilize PBSC [3, 4, 12, 14]. While many patients mobilize enough PBSC in one or two collections for more than one transplant, a significant number of patients do not achieve acceptable $CD34^+$ or CFU-GM numbers in PBSC products. Some investigators have reported that the time to recovery from the neutropenic nadir or thrombocytopenia separates patients who will have good PBSC collections from those who will not [12, 17, 18]. For example, patients with early hematologic recovery (<9 days) have higher numbers of progenitor cells collected than patients with prolonged aplasia after the same therapy [3, 9, 19-21]. This observation likely reflects an indirect measurement of stem cell reserve. Two studies from our institution using analysis by linear regression of the logarithm of $CD34^+$ cells collected found that lower age, marrow free of disease, lack of prior radiation and lower number of prior chemotherapy regimens were important factors influencing greater numbers of $CD34^+$ cells in collections [3, 4]. *Seong et al.* reported that previous pelvic radiotherapy, hypocellular marrow and refractory disease were associated with poor harvests of PBSC [22]. Similarly, *Haas et al.* reported that previous cytotoxic chemotherapy and radiation adversely affected the yield of $CD34^+$ cells [14] with each cycle of chemotherapy associated with an average decrease of 0.2×10^6 $CD34^+$ cells/kg per pheresis in nonirradiated patients, and large field radiotherapy reduced the collection yields by an average of 1.8×10^6 $CD34^+$ cells/kg. *Tricot et al.* reported a correlation between duration of exposure to previous chemotherapy, especially of alkylating agents, and mobilization yield in patients with multiple myeloma [12]. In that study, 91% of patients with exposure of <6 months reached $>5 \times 10^6$ $CD34^+$ cells/kg as compared to only 28% of patients with exposure >24 months. Recently, *Glaspy et al.* reported that frequency of previous chemotherapy, even with regimens which are not considered stem cell toxins, can decrease the number of $CD34^+$ cells harvested in patients with breast cancer. The median total number of $CD34^+$ cells obtained was significantly greater in the group receiving ≤4 cycles (11.7×10^6/kg) than in the group receiving ≥5 cycles (4.68×10^6/kg) ($p = 0.01$) [23]. However, these factors account for only about one-half of the interpatient variability and do not fully explain differences. Even in normal donors who are presumed to have normal marrow function and have not received any chemotherapy, the administration of granulocyte colony-stimulating factor (G-CSF) 5-16 μg/kg results in wide intersubject CD34 yields and poor yields in 5%-15% of donors [24-26].

Optimal Time for Collection of PBSC

The timing of PBSC collection is important in order to maximize the number of progenitors harvested. The most reliable time for harvesting hematopoietic stem cells is still under investigation. Pheresis usually is initiated 10 to 18 days after the administration of low to moderate dose chemotherapy. Following a chemotherapy-induced nadir, collections are usually initiated when the WBC count recovers to $>1 \times 10^9$/l [10, 27]. However, in one study investigators delayed collections until the WBC count was $>3 \times 10^9$/l [19]. Using this approach, a sufficient number of PBSC was collected in all patients with only a single leukapheresis. *Ho et al.* observed that maximum mobilization following CY, epirubicin and 5-fluorouracil with GM-CSF started consistently two days after the WBC count recovered to $>2 \times 10^9$/l after nadir, and remained elevated for four to five days [20]. Another study has suggested that a delay until the WBC count exceeds 10×10^9/l may be more optimum [28]. Thus, the best time to begin apheresis following chemotherapy still requires further study. We have observed poor $CD34^+$ cell yields in patients who recover WBC counts to $>1 \times 10^9$/l but remain platelet transfusion dependent (unpublished observations).

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Several studies reported that monitoring daily $CD34^+$ cell content in peripheral blood may also be useful to predict the best time to begin leukapheresis [21, 29-31]. *Siena et al.* recommended that pheresis begin when $CD34^+$ cells first appear in the circulation during recovery from intensive chemotherapy-induced pancytopenia [21]. *Haas et al.* reported that a $CD34^+$ cell count of at least 50 µl in peripheral blood was highly predictive for a yield greater than 2.5×10^6 $CD34^+$ cells/kg in a single apheresis [14]. *Passos-Coelho et al.* reported that percentage of peripheral blood $CD34^+$ cells ≥0.5% and of marrow $CD34^+$ cells ≥2.5% following CY and GM-CSF was highly predictive of successful mobilization yield [31]. In that study, $>2.9 \times 10^6$ $CD34^+$ cells/kg were collected after CY and GM-CSF mobilization in patients with greater than 2.5% $CD34^+$ cells in the bone marrow harvest before PBSC mobilization. All patients with a large number of collected PBSC had a peripheral blood $CD34^+$ cell percentage greater than 0.5% just prior to apheresis [31]. If a relatively rapid and inexpensive method for monitoring $CD34^+$ cells in peripheral blood were available, it would probably be worth monitoring every patient during recovery from chemotherapy in order to determine the optimum time for PBSC harvest.

Chemotherapy Plus Growth Factors For Collection of PBSC

Increased numbers of progenitor cells into peripheral blood were first observed after moderate-dose chemotherapy [28, 30-35]. Although chemotherapy alone can produce increases in the concentration of progenitors in peripheral blood, it is clear that the additional amplification observed when G-CSF or GM-CSF are given following chemotherapy allows more progenitors to be collected with fewer pheresis procedures. *Siena et al.* and *Sutherland et al.* reported that patients who received CY + GM-CSF-stimulated PBSC had more rapid and consistent platelet recoveries as compared with CY mobilized or steady-state PBSC alone [36, 37]. During the recovery phase from chemotherapy, the amplitude of the circulating stem cell concentration is related to the intensity of the myelosuppressive agents used [28, 30, 32, 36–39]. Intermediate-dose CY (4 g/m^2) and G-CSF had been used to collect stem cells on an outpatient basis, but higher doses of CY (7 g/m^2) are associated with higher stem cell yields [36–39]. Studies suggest that combinations of CY + etoposide (CE), CY + etoposide + cisplatin (CEP) or CY + Taxol® are probably more effective at stem cell mobilization than CY alone [11, 40, 41]. In one study from our institution, patients with advanced breast or ovarian cancer receiving CY (4 $g/m^2 \times 1$) and Taxol® (170 $mg/m^2 \times 1$) followed by G-CSF (10 µg/kg/day) had a median number of 13.02×10^6/kg $CD34^+$ cells (range 5.4-57.8) as compared to 6.39 (0.2-28) in patients receiving CY (4 $g/m^2 \times 1$) alone followed by G-CSF (16 µg/kg/day) ($p = 0.01$), suggesting that Taxol® added to CY followed by G-CSF was more effective than CY alone [41]. Similarly, *Raptis et al.* recently reported that addition of Taxol® (250 mg/m^2) to CY (3 g/m^2) and G-CSF yielded a median number of 16.22×10^6/kg $CD34^+$ cells as compared to 2.64×10^6/kg in patients receiving CY and G-CSF [42].

We have evaluated different chemotherapy regimens including CY alone, CE or CEP with G-CSF for mobilization of stem cells (unpublished observations). Results were measured using $CD34^+$ cells collected per kg body weight (Table 1). These results indicate that the combinations of CE or CEP are more effective at stem cell mobilization than CY alone, which agrees with other published results [43]. Furthermore, the addition of platinum to CY and etoposide causes further increase in the number of $CD34^+$ cells collected. CY and Taxol® appears to be as effective as CE in mobilizing PBSC (Table 1). The dose of growth factor utilized with chemotherapy may also be very important, although limited studies are available. We have compared G-CSF at 10 or 16 µg/kg with the regimens utilizing CY alone, CE or CEP (unpublished observations). There was an apparent dose response effect of G-CSF at 10 or 16 µg/kg in patients receiving CY, CE or CEP (Table 1).

Not all chemotherapy regimens produce adequate degrees of mobilization to be clinically useful. In general, agents that are known stem cell toxins such as BCNU, melphalan and thiotepa tend to be poor choices for mobilization although the Dexa-BEAM (dexamethasone, BCNU, etoposide, cytarabine, melphalan) regimen has been used successfully [28]. In general, chemotherapeutic agents which are effective for underlying tumors should be used for mobilization of stem cells. CY, etoposide, cisplatin, cytosine arabinoside (ara-C), mitoxantrone, ifosfamide and Taxol®

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Table 1. PBSC mobilization data with different chemotherapy regimens

Regimen	No. of Patients	G-CSF dose µg/kg	No. Collections median (range)	Mean daily $CD34^+ \times 10^6$/kg median (range)
CY	6	10	3 (1-7)	1.71 (0.65-6.28)
CY	9	16	4 (1-5)	2.13 (0.02-18.49)
CE	31	10	3 (1-10)	2.48 (0.19-55.41)
CE	23	16	2 (1-5)	3.35 (0.03-89.17)
CEP	16	10	2 (1-7)	8.25 (0.07-47.33)
CEP	13	16	2 (1-5)	10.18 (0.10-33.88)
CY + Taxol®	17	10	3 (1-7)	3.50 (0.8-28.9)

CY: Cyclophosphamide; CE: Cyclophosphamide + Etoposide; CEP: Cyclophosphamide + Etoposide + Cisplatin

with growth factors have been used for pre-transplant cytoreduction and PBSC mobilization.

The sequential or combined administration of interleukin 3 (IL-3) and G-CSF or GM-CSF following polychemotherapy has been shown to improve cell yields, compared to G-CSF or GM-CSF alone, as measured by numbers of CFU-GM, CFU-GEMM and BFU-E [33, 44-47]. *Haas et al.* demonstrated the efficacy of sequential IL-3/GM-CSF after ara-C and mitoxantrone in mobilizing PBSC with the capacity to reconstitute marrow function following myeloablative therapy [44]. *Hogge et al.* reported successful PBSC mobilization with IL-3 + GM-CSF following CY of 7 g/m^2 in nine heavily treated patients with relapsed or refractory Hodgkin's disease (HD), where a median of 9.8×10^6 $CD34^+$ cells/kg was collected [45]. In another study, seven patients received combined IL-3 (7.5 µg/kg/day) + G-CSF (5 µg/kg/day) following various chemotherapy regimens, and a median collection of 13×10^4 CFU-GM/kg was achieved [46]. In the above studies, the heterogeneity of patients in terms of disease, pre-treatment mobilization chemotherapy and the different CSF used makes firm conclusions regarding an optimal regimen difficult. Moreover, the time to recovery of neutrophils and platelets among patients transplanted with PBSC mobilized by different techniques does not appear to be different as long as a minimum number of $CD34^+$ cells/kg are given.

Growth Factors Alone For Collection of PBSC

Hematopoietic growth factors alone are often used to mobilize PBSC [48-52]. The exact mechanism by which CSF mobilize PBSC is unknown. They may act by causing the release of stem cells from marrow into blood. Because the increase in stem cells occurs several days after initiation of therapy, cytokines probably act in part by stimulating proliferation of stem cells either directly or by a secondary release of endogenous cytokines by stromal cells. G-CSF, GM-CSF and IL-3 each have been shown to be effective for mobilization of circulating PBSC. For example, a 63-fold increase in the numbers of circulating CFU-GMs has been observed after s.c. infusions of G-CSF [53]. When CSF are used for mobilization without prior chemotherapy, the peak output of $CD34^+$ cells occurs four to six days after initiating therapy [14, 21]. Further administration of recombinant human (rHu)G-CSF beyond seven days is not useful since progenitor cell concentrations in the peripheral blood fall beyond day 7 (*Dr. D. Stroncek*, personal communication).

Although no randomized studies are available, current data suggest that doses of CSF used for PBSC collections may be important. *Nademanee et al.* demonstrated a dose-response effect for G-CSF [52], with a seven-fold increase in the number of $CD34^+$ cells in the peripheral blood over the baseline value for 5 µg/kg/day and 28 times for 10 µg/kg/day. Similarly, there were 10- and 17-fold increases in CFU-GM over baseline for 5 µg/kg/day and 10 µg/kg/day of G-CSF, respectively. A shorter time to platelet and RBC transfusion independence when PBSC were used for transplantation has been reported with 10 µg/kg/day G-CSF compared to 5 µg/kg/day [52]. *Sheridan* has evaluated G-CSF in doses of 12 µg/kg/day or 24 µg/kg/day for mobilization and found superior collections of CFU-GM using the

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higher dose [54]. We have evaluated G-CSF in doses of 10-32 µg/kg/day (unpublished studies). The numbers of $CD34^+$ cells collected after 10-32 µg/kg/day appear to be superior to the reported studies utilizing 5 µg/kg/day in terms of fewer collections and faster engraftment kinetics. In studies utilizing 5 µg/kg/day of G-CSF, larger numbers of collections are required, and engraftment tends to be slower than in studies utilizing higher doses [52, 55]. *Zeller et al.* has evaluated G-CSF in doses of 10 µg/kg/day or 24 µg/kg/day for mobilization and found significantly superior collections of $CD34^+$ cells and MNC using the higher dose ($p = 0.02$ and 0.002, respectively) [56]. Patients sequentially treated in groups with 250 µg/m^2 or 125 µg/m^2 of GM-CSF were found to yield superior CFU-GM content in PBSC collections using the higher dose of GM-CSF [48]. The time to recovery of granulocytes, platelets and red cells was more rapid following transplantation of PBSC collected after a higher dose of GM-CSF. GM-CSF, while capable of mobilizing PBSC, is not well tolerated at doses >250 µg/m^2, which might be more effective, and this has somewhat limited the utility of the drug. These studies suggest that higher doses of cytokines can significantly increase the numbers of $CD34^+$ cells in leukapheresis products [52, 56], and further studies of G-CSF doses >10 µg/kg/day should be performed.

Hocker et al. compared the mobilization capacity of GM-CSF or G-CSF alone to pretreatment with 5 µg/kg day of IL-3 followed by G-CSF or GM-CSF combination and found the number of CFU-GM increased 10 to 101-fold with sequential therapy [57]. *Willerford et al.* reported a marked enhancement of PBSC mobilization in patients receiving a combination of G-CSF and GM-CSF, as compared with GM-CSF alone [58]. *Spitzer et al.*, however, reported that there were no statistically significant differences in regard to yield of $CD34^+$ cells and the duration of neutropenia or thrombocytopenia following high-dose chemotherapy between groups of patients receiving G-CSF alone or G-CSF + GM-CSF [59]. Combinations of G-CSF and GM-CSF do not appear to offer any advantage over G-CSF alone [58].

Other Cytokines for Collection of Stem Cells

PBSC mobilization using new cytokines such as PIXY (IL-3, GM-CSF fusion protein), stem cell factor (SCF) and flt2/flk3 protein currently are being evaluated. Although SCF has recently been shown to lead to the appearance of increased numbers of PBSC in the peripheral circulation, G-CSF needs to be given at the same time [55]. In a phase I/II study *Glaspy et al.* reported that administration of SCF in addition to G-CSF 10 µg/kg/day to patients with high risk breast cancer resulted in a four-fold increase in $CD34^+$ cells in the PBSC collections as compared to collections from patients receiving G-CSF alone [55]. Whether SCF administration will be associated with tolerable side effects which will permit its use remains to be determined. It is also not yet clear whether SCF added to optimum doses of G-CSF results in additional PBSC mobilization. PIXY has also been shown to be capable of mobilizing PBSC in one small dose escalation study [60]. Presently, G-CSF, because of its lack of toxicity and its well-known efficacy, is the most widely used cytokine for mobilization. The goal of the new cytokines or combinations should be invention of new regimens which consistently produce sufficient numbers of PBSC from a single leukapheresis or possibly one or two units of blood, in the majority of patients or normal donors.

Clinical Significance of Tumor Cells Contaminating PBSC Grafts

Recently, several reports have shown that PBSC infusions frequently are contaminated with tumor cells [61-65]. Circulating neoplastic cells may be present in patients with certain malignancies regardless of tumor involvement by the marrow. The possibility of infusing cancer cells into patients at the time of stem cell transplantation is known to occur in breast cancer, lymphomas, multiple myeloma (MM) and leukemias [61, 63, 64, 66]. *Brugger et al.* have shown that mobilization of PBSC can in some circumstances result in tumor cell recruitment into the peripheral blood in patients with small cell lung cancer or stage IV breast cancer [61]. Several studies have demonstrated that 50%-67% of patients with MM have malignant cells in circulation at the time of PBSC harvest [65, 67, 68]. The presence of these cells in circulation appears to correlate with disease activity and stage [67].

Despite the demonstration that leukemic cells infused with remission marrow can contribute to

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relapse, the overall significance of infused residual malignant cells to clinical relapse remains unknown [69]. It is, however, reasonable to assume that PBSC may contain occult malignant cells, and strategies should be developed to cope with this problem. Efforts to purge grafts of tumor cells have been carried out by incubation of grafts with chemotherapeutic drugs and tumor-specific monoclonal antibodies, and by positive selection for $CD34^+$ progenitor cells [70]. *Vescio et al.* reported that a highly purified collection of $CD34^+$ cells in patients with MM showed no myeloma specific antigen despite an assay sensitivity of one tumor cell in 2,500 to 44,000 normal cells [71]. While such efforts have led to reductions in tumor cell numbers present in PBSC grafts, the impact of such approaches on relapse or patient survival has not yet been determined [70]. It is also important to realize that any purging strategies result in some loss of stem cells. CD34 enrichment procedures have an average yield of only 50% [70]. The loss of such a large number of PBSC after selection could compromise the speed of engraftment. It is likely that novel purging methods are needed which are able to preserve the majority of progenitor cells.

Collection of Stem Cells from Normal Donors

Relatively few syngeneic or allogeneic transplants have been performed using PBSC due to concern about donor toxicities from growth factor administration and the theoretical increased risk of graft-versus-host-disease (GVHD) from the large number of allografted T cells. There is, however, increasing use of hematopoietic growth factors in healthy donors to facilitate the collection of granulocytes for transfusion or PBSC for transplantation [72-74]. Normal syngeneic donors underwent PBSC mobilization following G-CSF administration, and a median of 9.6×10^6 (range 1.6-12.6) $CD34^+$ cells/kg was obtained [73]. *Dreger* reported a median yield of 5.5×10^6 $CD34^+$ cells/kg from healthy donors following G-CSF (10 µg/kg/day) administration for five days [24]. In that study, the height of the $CD34^+$ cell peak was inversely correlated with the age of the donor. *Schmitz et al.* reported a median of 6.7×10^6 $CD34^+$ cells/kg (range 2.2-8.1) collected from healthy donors following G-CSF (10 µg/kg/day) [26]. Normal allograft donors underwent PBSC collection following G-CSF (16 µg/kg/day) for five days. The median $CD34^+$ cells/kg collected in two aphereses from healthy donors was 13.2×10^6 (range 6.9-21.6) [25]. As in patients, however, even healthy donors exhibit wide intersubject variation in the ability to mobilize PBSC that is not accounted for by age alone and obviously cannot be attributed to prior therapy. This large variability from donor to donor may confound the goal of collecting enough PBSC from only one to two units of whole blood. Preliminary studies have not shown an increase in acute GVHD compared to marrow encouraging future studies of allogeneic PBSC transplantation [25, 26, 75-76].

Future Prospects

Recently, a novel means of mobilizing a PBSC graft has been described. *Papayannopoulou et al.* have infused nonhuman primates with anti-alpha 4 integrin antibodies (Anti-VLA4), which are specific for adhesion molecules present on PBSC. Infusion of this antibody has resulted in a selective mobilization of PBSC (up to 200-fold) [77]. Such mobilization strategies, which alter the interaction of PBSC with bone marrow stromal cells, may decrease the contamination with tumor cells.

PBSC are increasingly utilized in lieu of marrow for stem cell support due to the ease of collection and the rapid kinetics of recovery relative to bone marrow. These results so far suggest that PBSC will likely supplant marrow for the majority of allogeneic and autologous transplants in the next five years.

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