Allogeneic peripheral blood stem cell transplantation

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Peripheral blood stem cell (PBSC) transplantation has been used effectively in the autologous setting for many years. More recently, allogeneic PBSC transplantation also has been used as a primary treatment for a variety of hematologic malignancies. This transition from autologous to allogeneic PBSC transplantation has several advantages. First, a healthy donor's PBSCs can be easily and efficiently mobilized and transfused to the recipient without major complications. These allogeneic PBSCs engraft rapidly. Second, allogeneic PBSCs may induce an immunologic graft-versus-tumor effect without a significant difference in the incidence of acute graft-versus-host disease (GVHD) as has been observed in allogeneic bone marrow transplant (BMT).1 Finally, the use of a large donor pool consisting of HLA-matched unrelated donors as well as HLA-mismatched donors allow for increased accessibility to allogeneic PBSC transplantation. This review focuses on the characteristics, mobilization, and engraftment of allogeneic PBSCs. In addition, specific donor issues and the incidence and significance of GVHD in the allogeneic PBSC recipient are discussed.

History
The existence of hematopoietic stem cells in the peripheral circulation was first suggested by Alexander Maximow in 1909.2 However, it was not until the 1950s that the existence of stem cells in the peripheral blood was proven when Brecher and Cronkite3 showed hematopoietic reconstitution in irradiated, myeloablated rats who were in crosscirculation with nonirradiated litter mates. Similar studies in dogs and baboons by Epstein et al.4 and Storb et al.5 respectively, confirmed the presence of marrow repopulating cells in larger animals and primates. In 1979, Goldman et al.6 reported the first human autologous PBSC transplant in a patient with CML. By 1988, it was shown that G-CSF and GM-CSF administration prior to PBSC leukapheresis led to the mobilization of large numbers of progenitor cells into the circulation and more rapid engraftment than that achieved using medullary bone marrow.7 This led some authorities to suggest that mobilized PBSCs would replace the use of bone marrow-derived stem cells by the year 2000.

The first successful allogeneic PBSC transplant used unmodified PBSCs collected from a normal donor following G-CSF mobilization and was performed by Dreger et al.8 in 1991 for the treatment of graft failure unresponsive to two marrow infusions. Shortly thereafter, Russel et al.9 reported the first use of G-CSF-mobilized PBSCs as a primary treatment for a patient with ALL in second remission. More recently, studies have shown that allogeneic PBSC transplantation using unrelated HLA-matched donors have outcomes similar to those achieved using related HLA-matched donors.10,11 In 1995, Stocksclader et al.11 studied a 41-year-old female with AML in second complete remission who underwent PBSC transplantation from an HLA-matched unrelated donor. The patient engrafted on day 19 and only displayed grade II acute GVHD (skin, liver), yet died on day 38 due to cerebral aspergillosis infection. In 1997, Massumoto et al.12 reported a case of an 11-year-old female with Fanconi’s anemia who received G-CSF-mobilized allogeneic PBSCs from an HLA-matched 41-year-old unrelated female donor. This patient was alive and well on day 191. At present, allogeneic PBSC transplantation is used primarily to treat relapse or graft failure or rejection after allogeneic BMT, to treat advanced or refractory hematologic malignancies, and when a selected donor is medically unable to donate bone marrow.13-15 Pediatric donors also have been considered as a source of cells for allogeneic PBSC transplantation. In 1996, Korbling et al.16 studied pediatric donors to determine whether they would tolerate G-CSF mobilization and leukapheresis. He found that up to 12µg/kg/day was well tolerated in the pediatric donor and that the only difficulty was main-
taining venous access and blood flow. Sufficient CD34+ cells were collected and engraftment characteristics and clinical outcome of five recipients were comparable to those achieved using adult PBSCs for transplantation.

**Donor Issues**

There are advantages for the donor of allogeneic PBSCs compared to bone marrow donation including the avoidance of general anesthesia, reduced need for red blood cell transfusions, and less pain. Presently, there are no well-defined contraindications to allogeneic PBSC donation in hematologically normal donors. However, the presence of inflammatory, autoimmune, rheumatologic, atherosclerotic, or cerebrovascular disease may be reasons to select another donor, if available. Some disadvantages for the donor include daily trips, daily injections, bone pain, flu-like symptoms, and possible central line placement. Only 25–30 percent of patients in need of a BMT have an HLA-matched family donor. Finding a matched unrelated donor also may be difficult due to the small but growing number of registries.

Beelen et al. have shown that a one antigen HLA mismatch leads to patient outcomes comparable to those observed following HLA-identical donations. His group prospectively compared allogeneic unmanipulated G-CSF mobilized PBSCs from HLA-phenotypically identical sibling donors to those obtained from partially matched related family donors. All patients showed timely and sustained engraftment with a lower rate of posttransplantation disease recurrence. Russell et al. also recently evaluated partially mismatched PBSC transplantation for high-risk hematologic malignancies and found results similar to Beelen's, along with a higher incidence of GVHD. The survival of the recipients was comparable to PBSC transplantation from fully matched donors.

**Mobilization Strategies**

**Cytokines**

**G-CSF.** In 1988, Socinski et al. documented that G-CSF stimulation expands the circulatory hematopoietic progenitor cell compartment in humans. In allogeneic PBSC transplantation, G-CSF is administered to the normal donor to obtain PBSCs for transplantation. When considering the use of G-CSF for mobilization of progenitor cells in normal donors, exposure should be as brief as possible to improve tolerability and minimize potential side effects such as bone pain and flu-like symptoms, yet enough progenitor cells need to be collected to ensure engraftment in the recipient.

Increased numbers of PBSCs have been obtained from normal donors following administration of G-CSF at doses of 2–16 μg/kg/day. G-CSF has been shown to have a dose-dependent effect, and many studies have shown that 10 μg/kg/day is superior to 5 μg/kg/day. However, studies using both high and low doses of G-CSF have shown promising results. Matsunaga et al. treated normal donors with 2.5 μg/kg/day for 6 days and then 5 μg/kg/day for the next 4 days. CFU-GM peaked on day 6. In contradistinction, Majolino et al. administered 16 μg/kg/day for 4 days to normal donors and obtained a sufficient number of PBSCs for allogeneic transplants without minimal side effects. This higher dose regimen was able to decrease the total number of days the donors were exposed to G-CSF and the total number of aphereses required. It appears that most studies have found peak levels of PBSCs 4 to 5 days after beginning daily G-CSF treatment. The exact optimum dose and scheduling of G-CSF has not been determined and may depend on other factors such as age, weight, and overall health of the donor.

**GM-CSF.** Although G-CSF has been used in most allogeneic PBSC transplantation mobilization regimens, some investigators have studied GM-CSF alone or in combination with G-CSF. Ho et al. found that a combination of G-CSF and GM-CSF stimulated a significantly higher proportion of pluripotent CD34+ subsets than G-CSF alone, whereas GM-CSF alone was not as efficient in mobilizing an adequate number of CD34+ cells. The yield of progenitor cells obtained with the combination of G-CSF and GM-CSF approaches that of fetal cord blood, which is known to contain a higher number of primitive pluripotent stem cells. Similarly, Lane et al. found that a combination of G-CSF and GM-CSF showed equivalent mobilization of CD34+ cells and CFU-GM cells as G-CSF alone, but a greater number of primitive progenitor cells (CD34+/CD38−) were mobilized with the combination of drugs. It is not known whether mobilization of these primitive cells is associated with more rapid engraftment. In addition, the total number of CD34+ cells may not correlate directly with clinical reconstitution.

**Safety**

Even though there are advantages in donating cytokine-mobilized PBSCs over bone marrow, there have
been concerns raised about the potential for short- and long-term adverse effects of G-CSF administration to hematologically "normal" donors. Anderlini et al. studied 40 normal donors receiving 12 μg/kg/day of G-CSF and 33 normal bone marrow donors and compared clinical toxicity and laboratory effects. Minimal or mild adverse reactions were reported in 98 percent of the PBSC donors, including bone pain (controlled by mild analgesics), headaches, nausea, fatigue, local reaction, and fever. None of the donors discontinued the G-CSF prematurely. Most adverse side effects resolved within 7 days after completion of the G-CSF treatment. There are some individual reports of rare, serious (splenic rupture, anaphylactoid reaction) adverse effects that have been associated with the administration of G-CSF to presumed healthy individuals.

The long-term safety of G-CSF in normal donors remains to be determined. The longest follow up reported is 4 years by Korbling et al., and no adverse long-term effects have been identified. Donor safety concerns will become even more important if unrelated donors are used. Recently, a possibility of increased risk of acute leukemia has been discussed. Leukemic transformation associated with G-CSF administration has been reported in patients with aplastic anemia and congenital neutropenia. Long-term follow-up studies will be needed to assess the risk of clinically important side effects in allogeneic PBSC donors after receiving G-CSF and whether these risks are outweighed by the potential benefits to the donor. It has been estimated that more than 2000 donors would need to be followed for 10 years to detect nine cases of excess leukemia due to G-CSF.

Laboratory effects and toxicity

Common laboratory values altered by use of G-CSF are the WBC (mean 42.7 × 10^9/L), platelets, neutrophils, lymphocytes, monocytes, LDH, and potassium. The increases in WBCs, neutrophils, and lymphocytes are profound, yet these values usually normalize within 2 to 3 weeks after cessation of G-CSF. An increase in LDH and Mg++ are consistent with expansion of the marrow pool of progenitor cells. K+ slightly decreases with G-CSF administration and may need to be replaced in some patients. Overall, the side effects and short-term safety of G-CSF in normal donors are acceptable, yet careful monitoring of donor hematopoiesis and chemistry profiles is necessary.

Large Volume Leukapheresis

It is possible to obtain stem cells from the peripheral blood of normal donors without G-CSF mobilization, but greater than ten leukaphereses may be needed, which is impractical and which increases the risks to the donor. Since 1990, large volume leukapheresis (LVL) has become the main approach to PBSC harvest. LVL is defined as the processing of greater than three blood volumes or up to 15-25 liters of blood in one sitting. This process is safely and easily accomplished, results in a predictable collection of mononuclear cells (MNC) based on preapheresis MNC counts, and has replaced standard apheresis procedures for the normal donor receiving G-CSF. Only 1 to 2 LVL procedures are needed to obtain an adequate number of progenitor cells. Moreover, with an increased daily dose of G-CSF (10-15 μg/kg/day), the number of leukaphereses may often be reduced to a single LVL procedure. Majolino et al. reported that administering 16 μg/kg/day of G-CSF and performing leukapheresis on days 4 and 5 resulted in collection of an adequate number of progenitor cells. These findings imply that a larger dose of G-CSF (if tolerated) for a shorter amount of time may yield enough progenitor cells with only 1 to 2 LVLs, thereby decreasing the risk of the procedure to the donor.

Laboratory effects of LVL in donors

Transient thrombocytopenia occurs in the donor and is presumably apheresis-related, but a G-CSF effect cannot be ruled out. Okamoto et al. reported prolonged grade III thrombocytopenia (WHO toxicity criteria) in a hematologically normal donor after G-CSF administration and leukapheresis. The donor received 5 μg/kg of G-CSF every 12 hours for 5 days. The donor's WBCs rose from 51,000/μL to 91,000/μL, and platelets decreased slightly but remained within normal limits. On the fourth day of G-CSF treatment and after the second leukapheresis, the platelet count dropped from 179,000/μL to 65,000/μL and reached the lowest value of 47,000/μL on day 7. No bleeding episodes were noted and the platelets returned to normal with one week.

Novotny et al. recently reported 48 healthy donors who underwent 1 to 4 PBSC aphereses procedures following 4 days of G-CSF treatment in which a median of 55.9 × 10^9 lymphocytes were collected. The donor mean peripheral lymphocyte counts decreased from premobilization values of 2.31 × 10^9/L to 1.31 × 10^9/L at a median of 1 month and 1.53 × 10^9/L at a median of 11 months. This decrease in peripheral lymphocytes correlated with the number of lymphocytes removed and the
number of aphereses. Neutrophil counts returned to normal within 1 month; monocyte counts were significantly decreased at 1 month but not at 11 months.

Characterization of Mobilized PBSCs

G-CSF-mobilized autologous and allogeneic PBSCs contain both committed and primitive hematopoietic precursors that show phenotypic and functional differences upon comparison to bone marrow progenitor cells. Recently, Stroncek et al. measured the quantity, quality, composition, and variability of PBSCs collected from 150 healthy donors given G-CSF (5, 7.5, or 10 μg/kg/day) for 5 days. The average quantities of the different cellular components were as follows: WBCs, 35.0 ± 16.4 × 10⁹; mononuclear cells, 33.3 ± 14.4 × 10⁹; CD34+ cells, 412 ± 287 × 10⁹; neutrophils, 1.71 ± 3.59 × 10⁹; red blood cells, 7.2 ± 4.0 mL; and platelets, 480 ± 110 × 10⁹. The PBSC components collected from donors receiving 7.5 or 10 μg/kg/day G-CSF contained significantly more CD34+ cells than those from donors given 5 μg/kg/day of G-CSF.

Tjonnø-Jørg et al. studied in vitro colony formation and the immunophenotypic of CD34+ cells mobilized by G-CSF in healthy PBSC donors and found a larger number of myeloid-associated cell surface markers (CD34+, CD33+, CD11b+) as well as colony-forming cells with increased clonogenicity when compared to bone marrow cells. These findings may account for the accelerated marrow reconstitution and more rapid engraftment noted with the use of these cells. Korbling et al. also found a larger number of primitive CD34+ subsets (CD34+Thy-1dim CD38-, CD34+Thy-1dim) in mobilized PBSCs versus bone marrow cells. In addition, there is differential expression of adhesion molecules on CD34+ cells in PBSCs, which may also be a factor accounting for hastened reconstitution. Dereksen et al. suggested that molecules such as L-selectin on CD34+ cells are important for either homing of CD34+ cells to the bone marrow or in the regulation of hematopoiesis. Additional data suggests that adhesion molecules may be expressed differentially between bone marrow cells and PBSCs. However, Lemoli et al. state that the PBSCs and bone marrow cells are the same in content, yet the PBSCs are higher in cellularity with an increasing number of early hematopoietic progenitor cells than bone marrow harvests. It remains to be clearly demonstrated whether there is a true qualitative difference between PBSCs and bone marrow cells, and whether this difference is due to the G-CSF, which leads to mobilization, proliferation, and differentiation of precursor cells. It is now well estab-

lished that PBSCs result in more rapid engraftment than bone marrow-derived stem cells, which is advantageous as it leads to reduced antibiotic days, number of transfusions, and shortened hospital stays.  

CD34+ Selection/T-Cell Depletion

There are many methods available to enrich for CD34+ cells from both bone marrow and PBSC compartments using positive and/or negative selection steps. In doing so, the T-cell content of the PBSC component, which is 1 to 2 logs greater than bone marrow products, is indirectly reduced, potentially decreasing the risk of GVHD. In addition, the volume of cryopreservation media and the number of undesired cells can be minimized without compromising the component efficiency. The most commonly described methods for CD34+ selection are (a) monoclonal antibodies against CD34+ cells using the biotin-avidin system, (b) cell selection flasks coated with anti-CD34+ antibody; and (c) the immunomagnetic bead method. Counterflow centrifugal elutriation to concentrate CD34+ cells and CFU-GM progenitors on a large scale also have been described.  

Processing and Cryopreservation

Although allogeneic PBSCs may be infused fresh and the potential for cell loss and the expense of freezing can be avoided, it may be beneficial to cryopreserve the cells in order to ensure adequate PBSC quantity prior to the myeloablation of the recipient. Indeed, it is possible to cryopreserve the PBSCs and achieve good recovery of the cells at a later date for infusion into the recipient. Studies indicate that engraftment characteristics of the cryopreserved stem cells are not significantly different than fresh PBSCs. Cryopreservation provides flexibility in the time to transplant the recipient and may be even more useful if unrelated donor PBSCs are used.
the apheresis product is put through manual centrifugation to remove the excess plasma. Cellular cryoprotectants (DMSO or DMSO and HES) are added and the product is frozen in a rate-controlled freezer and stored in liquid nitrogen.66-69 Recent studies have suggested an alternative to the conventional procedure using non-rate-controlled freezing in a -80°C freezer. This simplified method appears to be more efficient, less expensive, and can offer long-term storage for at least 5 years with no significant decrease in recovery of hematopoietic progenitor cells.70

Dose

The most reliable in vitro indicator of hematopoietic adequacy in autologous PBSC transplants is the number of CD34+ cells/kg of recipient body weight.51 However, there is not yet agreement on the optimum number of CD34+ cells needed to ensure engraftment. A minimum of 5 x 10^6 CD34+ cells/kg has been recommended to ensure engraftment in the recipient.52 Storb et al.53 found that 1 x 10^8 mononuclear cells/kg, 5 x 10^4 CFU-GM/kg, and 2 x 10^6 CD34+ cells/kg are needed for successful engraftment in allogeneic BMT. This minimum has been applied to allogeneic PBSC transplantation. Some authors disagree and recommend even higher minimum doses of CD34+ cells.54 Further investigation is needed to define the optimum dose of CD34+ cells to ensure engraftment in allogeneic PBSC transplantation.

Flow cytometric analysis, an accurate and rapid quantification technique, may be used to determine if an adequate number of CD34+ cells are present in the peripheral blood of the donor and when to begin leukapheresis procedures.55

Outcomes

Table 1 summarizes representative studies of short-
and long-term engraftment and acute and chronic GVHD in allogeneic PBSC transplants.

**Engraftment**

Short-term engraftment in hematopoietic transplantation is defined by the number of days that elapse post-transplant until the recipient achieves an absolute neutrophil count of \( > 0.5 \times 10^9/L \) and a platelet count of \( > 20 \times 10^9/L \). Most studies have shown more rapid engraftment, defined above, with allogeneic PBSC transplants than with allogeneic BMTs. Methotrexate is commonly administered posttransplant as GVHD prophylaxis. Some authors suggest that methotrexate causes a delay in engraftment, yet this has not been a problem on a large scale.\(^{56,57}\)

Long-term engraftment in allogeneic transplantation may be demonstrated by PCR amplification of short tandem repeats, which are repetitive sequences of 3 to 7 base pairs of DNA. Documenting the chimeric status of the patient may be helpful in assessing graft rejection and disease relapse. There is no significant difference in long-term engraftment between allogeneic PBSC transplantation and BMT. The longest follow-up with chimerism studies of allogeneic PBSC transplants is 2 years. Thus far, late graft failures are rare.\(^{58-61}\)

**Acute GVHD**

Controversy exists over whether ex-vivo T-cell depletion (or CD34+ selection) is needed to prevent GVHD and whether it contributes to increased graft failure and relapse by diminishing the GVL effect. Most patients receive a combination of cyclosporine with either methotrexate or prednisone as prophylaxis against GVHD. In a large study by Bensinger et al.,\(^{62}\) the estimated risk of developing acute GVHD grades II-IV was 37 percent for allogeneic PBSC transplant patients and 56 percent for a historical BMT group (p = 0.18). Overall, most studies have found that approximately 30 percent of patients develop acute grades I-IV GVHD, which is not significantly different from allogeneic BMT patients despite the infusion of 1 log to 2 logs more T cells with PBSC transplantation.\(^{1}\) Interestingly, Zeng et al.\(^{63}\) proposes that there is no significant difference in GVHD between PBSC transplantation and BMT because the G-CSF given to the donor pretransplant may alter the T-cell function in the PBSC product.

**Chronic GVHD**

Recent studies have suggested that allogeneic PBSC transplants may result in an increased risk of chronic GVHD, although the exact incidence of chronic GVHD is not known. Majolice et al.\(^{64}\) found that six of nine patients developed chronic GVHD, with some patients developing chronic GVHD de novo without signs of acute GVHD. The author proposes that the low incidence of acute GVHD is counterbalanced by an increase in chronic GVHD and states that cyclosporine may be less effective in treating chronic GVHD. Storek et al.\(^{65}\) studied 74 patients for 2 years and found that 32 percent of BMT patients developed chronic GVHD, whereas 54 percent of PBSC transplant patients developed chronic GVHD. This author suggests G-CSF mobilization of the PBSCs may alter the function of the T-cells and that this alteration may be beneficial. Early posttransplantation in preventing acute GVHD but may fade and lead to increased chronic GVHD. Also, 2 logs more of monocytes are present in PBSC grafts than in bone marrow; these cells are capable of suppressing T cells. In fact, a lower incidence of relapse is associated with patients who develop chronic GVHD, most likely due to the number of T cells causing a GVL effect.

**Graft-versus-leukemia**

CD34+ selection is important when considering GVHD risk. With CD34+ selection it is possible to decrease the T-cell count to as low as \( 0.03 \times 10^6/kg \), but Link et al.\(^{66}\) suggests that a minimum of \( 1 \times 10^5 \) T cells are needed to obtain a GVL effect as well as to prevent graft rejection and/or failure. In contrast, Schmitz et al.\(^{57}\) performed primary PBSC transplants on eight patients without T-cell depletion or CD34+ selection, and obtained engraftment in all patients with no signs of GVHD in three of eight patients and minimal GVHD in four of eight patients. Only one developed grade III GVHD secondary to refusing cyclosporine therapy and subsequently relapsed. The data are limited, but suggest that transfusion of unmanipulated PBSC products may be feasible and can result in long-term engraftment without detrimental GVHD and possibly better GVL effect. Further studies are needed to delineate the exact role of T cells in the PBSC product and whether there is a significant difference in outcome when the PBSC product is not manipulated.

**Conclusion**

Many authorities now agree that PBSCs are an acceptable source of progenitor cells for allogeneic transplantation and may likely offer an alternative to allogeneic BMT in the near future. Many advantages are readily apparent, including, for the donor, the avoidance of gen-
eral anesthesia and a possible lower risk of complication involved by using leukapheresis. Potentially, the donor pool can be expanded if unrelated, pediatric, and older individuals (up to 70 years) are included. Along these lines, the PBSC product can be effectively cryopreserved and stored for long periods.

Allogeneic PBSC transplantation is advantageous to the patient for many reasons. PBSCs lead to a more rapid engraftment than conventional BM grafts, thus leading to a lower incidence and severity of infectious complications and less use of antibiotics, shorter hospital stay, and less transfusion support. Surprisingly, acute GVHD has been less than expected even with the increased number of T cells infused, but chronic GVHD appears to be increased in some reports. Some data suggest that the PBSC graft may lead to a more pronounced GVL effect secondary to the increased number of T cells, or even more likely, the high number of T cells decreases the risk of graft failure, especially if some HLA disparity is present.

Efforts should be made through future studies to accurately define the precise indications for allogeneic PBSC transplantation. Studies should focus on defining the qualitative differences between PBSCs and bone marrow, the optimum dose of G-CSF, and the CD34+ selection method that is most effective. This in turn may result in growth factor regimes that mobilize enough PBSCs from only 1 to 2 units of blood. Long-term donor safety issues concerning G-CSF are important especially if unrelated donors are incorporated into the donor pool.

References


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