Transplantation of Allogeneic Peripheral Blood Stem Cells Mobilized by Recombinant Human Granulocyte Colony Stimulating Factor

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Concise Review

Transplantation of Allogeneic Peripheral Blood Stem Cells Mobilized by Recombinant Human Granulocyte Colony Stimulating Factor

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Key Words. Allogeneic • Transplantation • Peripheral blood stem cells

Abstract. Recombinant G-CSF has been given to over 150 normal donors for the collection of allogeneic or syngeneic peripheral blood stem cells (PBSC). G-CSF was found to be well-tolerated with mild-moderate bone pain, edema and mild thrombocytopenia being the observed side effects. To date, approximately 90 unmodified primary PBSC transplants from HLA-identical related donors have been performed with engraftment that is, in general, considerably more rapid than marrow. Acute graft-versus-host-disease (GVHD), grades II-IV occurred in 47% of patients and grades III-IV in 17%. Despite the infusion of one to two logs more T cells, these results are not remarkably different than would be expected with marrow transplantation. There have also been successful reports of using G-CSF mobilized allogeneic PBSC following second transplants for graft rejection or relapse. Allogeneic PBSC have been infused without reconditioning for correction of graft failure and unmodified or CD34 selected PBSC have also been given with marrow to augment the dose of hematopoietic cells. Further studies are needed to define the role of allogeneic PBSC for transplantation, refine PBSC mobilization and collection techniques and to evaluate the long-term effects of cytokines in normal donors. *Stem Cells 1996;14:90-105*

Introduction

It has long been known that hematopoietic precursors circulate in the peripheral blood [1] and can be utilized for autologous or allogeneic transplantation [2, 3]. In man, low, steady-state concentration of these cells has precluded harvesting of useful numbers within a practical number of apheresis procedures given the current technology [4]. In one early attempt at allogeneic peripheral blood stem cell (PBSC) transplantation, a patient received a series of 10 buffy coat infusions that were T-depleted after collection from a donor who did not receive a growth factor [5]. Although neutrophils recovered by day 11 and a marrow examination demonstrated trilineage engraftment on day 27, death from infection occurred on day 32, which prevented evaluation of platelet engraftment and graft-versus-host-disease (GVHD). Clearly, 10 two to four-hour apheresis procedures are neither practical nor cost-effective for procuring hematopoietic stem cells from normal donors on a routine basis. However, PBSC collected in one to three apheresis procedures from patients with malignant disease following the administration of a colony stimulating factor can produce rapid and durable autologous hematopoietic reconstitution when infused after myeloablative therapy [6, 7]. These observations led to the demonstration that the administration of a recombinant growth factor to normal individuals increased the number of hematopoietic precursors in the peripheral blood where they could be collected in large quantities and used for syngeneic and potentially for allogeneic transplantation [8-10]. Syngeneic transplants were ideal for initial trials of PBSC collected from normal donors as the infusion of large numbers of syngeneic T cells does not cause severe GVHD.

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Several successful syngeneic PBSC transplants have been performed with durable engraftment and follow-up extending beyond two years [8]. The administration of relatively high doses of recombinant human (rHu) G-CSF to normal syngeneic PBSC and allogeneic granulocyte donors has been well-tolerated without measurable effects over four years [8, 11, 12].

The first successful allogeneic transplant using unmodified PBSC collected from a normal donor following the administration of G-CSF was performed by *Dreger et al.* in 1991 for the treatment of graft failure unresponsive to two marrow infusions [13]. In this case the donor did not wish to undergo a third marrow aspiration. This patient is currently alive and well four years after PBSC infusion. The first primary allogeneic transplant utilizing PBSC collected after G-CSF was reported by *Russell et al.* in 1993 [14]. This patient had acute lymphocytic leukemia (ALL) in second remission and was treated with cyclophosphamide and TBI followed by unmodified PBSC and GVHD prophylaxis with cyclosporine and methotrexate. He engrafted promptly and is alive and well without acute or chronic GVHD more than 24 months after transplantation. Peripheral blood stem cells were used in this case because the donor was at high risk for anesthetic complications due to morbid obesity. These two reports of allogeneic [13, 14] and the success of syngeneic PBSC transplants [8] have led to a rapid application of this technology. Two recent editorials have summarized the current status of using G-CSF mobilized PBSC for allografting [15, 16].

The results of allogeneic PBSC transplantation to date suggest that this technique can produce substantially more rapid engraftment than observed with marrow. Further, contrary to widespread expectations, acute GVHD has not been intolerable, even with unmanipulated PBSC containing many more T cells than are present in a normal marrow graft. There have also been successful reports of using allogeneic PBSC following second transplants for graft rejection or relapse. Allogeneic PBSC have been infused without reconditioning for correction of graft failure. Allogeneic PBSC have also been given with marrow to augment the dose of hematopoietic cells. The purpose of this manuscript is to review the current results of allogeneic PBSC transplantation and to speculate on the future role of this technology.

Characteristics of Growth Factor Mobilized Peripheral Blood Stem Cells

Phenotypic and Functional Characteristics

The phenotypic characteristics of PBSC collected after growth factor administration have been reviewed by *Rice* and *Reiffers*, who concluded that a mixture of primitive and committed hematopoietic precursors were present that were not remarkably different from marrow [17]. Antigenic analysis of $CD34^+$ PBSC revealed that these cells co-expressed a variety of differentiation antigens with properties that were similar to marrow-derived $CD34^+$ cells including subpopulations which were: $CD34^+/CD33^-$, $CD34^+/HLADR^-$, $CD34^+/HLADR^+$ and $CD34^+/CD33^+$ [18, 19].

In humans, G-CSF mobilizes cells which are resistant to 5-fluorouracil (5-FU) in culture and contain a mixture of hematopoietic progenitor cells similar in characteristics to marrow-derived stem cells [20]. These 5-FU-resistant PBSC are also capable of cytokine-mediated expansion in vitro [21]. These data provide circumstantial evidence that G-CSF-mobilized PBSC contain primitive hematopoietic precursors.

Recently published reports as well as our own preliminary data indicate that $CD34^+$ cells collected from the peripheral blood after G-CSF differ from those in marrow in terms of adhesion molecule expression, proliferative potential and cytokine response [22, 23]. Any and all of these characteristics may provide some advantage to G-CSF-mobilized $CD34^+$ cells in terms of being more efficient in homing to the microenvironment and being more responsive to the signals produced there. Additional studies are needed to define qualitative or quantitative differences between marrow and PBSC that influence engraftment kinetics and determine if these differences are dependent on the specific growth factor or combination of growth factors used for mobilization.

Accessory Cell Content of PBSC

Rapid engraftment of autologous or allogeneic PBSC may not be due entirely to increased numbers or altered function of $CD34^+$ cells. Another potential explanation for the rapid engraftment seen with G-CSF-mobilized PBSC is the presence of accessory cells which may be increased in number or qualitatively more capable of enhancing the milieu of the

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Table 1. Cytokine concentration (pg/ml) in supernatant of four-day cultures[a]

	IL-2	IL-6	M-CSF	G-CSF
G-PBC Alone	121	49	277	11
Stroma Alone	18	489	50	12
Stroma + MMC	103	15,290	6	54
Stroma + G-PBC	331	120,000	6	476

[a]Primary stromal cultures were grown to confluency and then used for study. The G-CSF stimulated peripheral blood cells (G-PBC) and marrow mononuclear cells (MMC) were obtained from the same donor and added at 3×10^6 per ml to the established stroma. M-CSF = Macrophage-Colony Stimulating Factor; IL-2 = Interleukin 2; IL-6 = Interleukin 6; G-CSF = Granulocyte-Colony Stimulating Factor.

hematopoietic microenvironment. Initial studies designed to test this hypothesis evaluated the generation of cytokines by stromal cells cocultured with marrow mononuclear cells or mononuclear cells collected from the blood after the administration of G-CSF or with marrow mononuclear cells (Table 1). Interleukin 6 (IL-6) and G-CSF levels were increased when marrow or G-CSF stimulated peripheral blood mononuclear cells were added to stroma, but the increase was 10-fold greater with the latter.

T Cell Content of PBSC

The T cell content of normal PBSC as compared to marrow is shown in Table 2 [24, 25]. There are 1-2 logs more T cells infused with PBSC as compared to marrow. We did not find an increase in T cells in the peripheral blood following the administration of 16 µg/kg of G-CSF [24]. However, *Matsunaga et al.* administered G-CSF 2.5 µg/kg for six consecutive days followed by 5 µg/kg for the following four days and found a significant increase in circulating T cells by day 8 [26]. *Dreger et al.* found that PBSC contained seven times more T cells and 20 times more natural killer (NK) cells than marrow [9]. *Körbling et al.* found a 1.0-1.4 log increase in T cell subsets in PBSC harvests as compared to marrow [27].

Table 2. Cellular composition of marrow and PBSC[a]

Cell Phenotype	Marrow $n = 18$ $\times 10^6$/kg[b]	PBSC[a] $n = 25$ $\times 10^6$/kg[b]
$CD34^+$	4.1 (0.6-5.7)	11.1 (6.9-21.0)
$CD3^+$	31 (10-65)	419 (81-846)
$CD4^+$	19 (1-47)	224 (45-975)
$CD8^+$	10 (1-24)	126 (73-355)

[a]PBSC = Peripheral Blood Stem Cells
[b]$\times 10^6$/kg of recipient body weight

These data show that the numbers of T cells infused will be directly related to the number of apheresis collections infused. Thus, efforts to increase the $CD34^+$ content of each collection in order to achieve the desired number with the fewest collections will reduce the number of T cells infused in the graft.

Immunotherapeutic Potential of PBSC

Neubauer et al. demonstrated that precursor cells capable of acquiring IL-2-inducible lymphokine-activated killer (LAK) activity were present in G-CSF-mobilized PBSC and that LAK activity could be detected in the blood of all patients post-transplant [28]. *Verbik et al.* evaluated the frequency of cytotoxic effector cells, including LAK cell precursors following GM-CSF administration and concluded that PBSC contained considerable numbers of precursor cells capable of generating LAK cells [29]. Their results also suggested that cells from earlier collections containing higher numbers of LAK precursor cells would be best suited for ex vivo manipulations as compared to a later more optimal time for $CD34^+$ cell collections. These observations have little importance at the present time for allogeneic PBSC transplantation. However, such observations could become important if the cell populations that cause GVHD could be separated from the cells that are responsible for a graft-versus-tumor effect.

Allogeneic PBSC Transplants in Animals

Successful allogeneic PBSC transplants have been performed in the canine model using peripheral blood mononuclear cells collected without chemotherapy or growth factor administration

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[3, 30]. *Molineux et al.* performed the first study of G-CSF mobilized PBSC in an animal model where syngeneic transplants were carried out in mice using sex-mismatched donors to evaluate donor/host chimerism [31]. They were able to demonstrate durable marrow repopulation with donor cells up to 192 days after transplant. However, there are no published reports describing the use of non-syngeneic donors in the mouse model to evaluate graft rejection or GVHD.

In murine models, stem cell factor (SCF) and G-CSF or a combination of the two increases the concentration of colony forming units-spleen (CFU-S) in the peripheral blood [32-37]. In the mouse, IL-8 also mobilizes PBSC which have long-term marrow repopulating ability [38]. G-CSF, SCF and more recently IL-8 have been shown to increase the number of hematopoietic precursor cells in the peripheral blood of baboons [39, 40]. Studies in dogs showed canine G-CSF and canine SCF to be synergistic with regard to mobilizing transplantable PBSC from the peripheral blood [41]. Peripheral blood progenitor cells mobilized by canine SCF have also been shown to be targets for gene transduction with long-term persistence demonstrated after transplantation [42].

Based on data generated in the canine autologous transplant model, we have used recombinant canine G-CSF alone and in combination with recombinant canine SCF to determine mobilization strategies that would result in successful engraftment with allogeneic PBSC [43]. Animals were conditioned with 9.2 Gy TBI and no post-transplant immunosuppression was administered. In both the dog leukocyte antigen (DLA)-identical and the DLA-haploidentical settings, all animals engrafted promptly which was documented by variable number tandem repeat probes and, in the cases of sex-mismatched transplants, by cytogenetics. The rate of engraftment of DLA haploidentical allogeneic PBSC was significantly greater than observed with marrow alone where 50% of dogs historically failed to engraft or rejected. However, all nine dogs that received haploidentical transplants were euthanized because of hyperacute GVHD. In the nine dogs that received DLA-identical transplants, eight dogs developed GVHD which was transient in five and fatal in three [43, 44]. The incidence and severity of acute GVHD was no greater but not less than would have been expected using marrow from DLA-identical donors where no post-transplant immunosuppression was used. In several animals, the cytokine-mobilized donor cells failed to proliferate in response to mismatched allogeneic cells in mixed leukocyte culture and did not stimulate mismatched allogeneic lymphocytes as well as before cytokine treatment (unpublished observation). These findings are consistent with those made with human cells after in vitro treatment with G-CSF [45].

The only other reported animal model of the use of allogeneic G-CSF-mobilized PBSC is in rabbits. Adult outbred red Burgundy rabbits were used as donors and New Zealand white rabbits of the opposite sex were used as recipients [46]. It was demonstrated that PBSC mobilized with human G-CSF can engraft across major histocompatibility barriers with an incidence of GVHD that was no different from that of unmanipulated bone marrow.

Taken together, these limited data from animal models suggest that allogeneic PBSC engraft better than marrow and cause no more acute GVHD despite the infusion of large numbers of lymphocytes. The increased rate of engraftment observed in the mismatched situation could be due to the added stem cells or to the increased number of lymphocytes infused. Whether or not growth factor-mobilized PBSC are immunologically different than marrow remains to be determined in the various animal models available.

Allogeneic Peripheral Blood Stem Cell Transplant in Humans

Collection of PBSC after the Administration of Recombinant Growth Factors in Normal Donors

There are extensive data from the autologous PBSC transplant experience to predict toxicities likely to be observed in normal donors receiving recombinant growth factors followed by one or more apheresis procedures [6, 7]. There are also accumulating data on engraftment following autologous and syngeneic PBSC transplants that, by extrapolation, may assist in making decisions about cell dose requirements for allogeneic PBSC transplantation [6-8].

Toxicities of G-CSF

Although other growth factors have been evaluated in animals and in humans undergoing

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autologous PBSC transplantation, G-CSF has been the predominant drug evaluated in normal donors for granulocyte and PBSC harvesting. G-CSF was chosen for evaluation in normal donors because of its low toxicity profile in patients receiving autologous PBSC transplants. Administration of G-CSF in doses up to 16 μg/kg/day has been well tolerated in normal donors with bone pain, flu-like symptoms and myalgias being the major side effects [8-14, 47-75]. There are no reports of discontinuation of G-CSF due to immediate side effects in normal PBSC donors. Symptoms and the granulocytosis induced are, in general, reversed within 48 h of discontinuing the drug.

In donors undergoing daily administration of G-CSF and daily granulocyte collections for 7-14 days there was a significant decrement in platelet levels when compared with patients undergoing the same collection procedures without the administration of G-CSF [11]. In patients undergoing one or two apheresis procedures for the collection of PBSC, we have not observed platelet decrements to levels below 100×10^9/l. However, all six allogeneic donors who underwent four consecutive daily collections of PBSC following 16 μg/kg/day of G-CSF had decrements of circulating platelets to 100×10^9/l or less and 3/6 had decrements to levels less than 50×10^9/l necessitating reinfusion of collected platelets after centrifugation of the PBSC product. Platelet decrements in donors may also be exacerbated by relatively high centrifugation speeds resulting in a higher yield of platelets in the PBSC collection product. It is known from phase I studies of G-CSF administration to patients who were not apheresed that platelet levels decrease [42]. Similar findings of platelet decrements have also been observed following human or canine GM-CSF administration in a dog model [76]. However, the exact mechanisms of thrombocytopenia following G-CSF facilitated PBSC or granulocyte harvest are unknown.

Comparison of the Risks of Bone Marrow versus PBSC Harvests

There is concern that the administration of G-CSF to normal individuals could result in future disorders of hematopoiesis including leukemic transformation. These potential complications of G-CSF administration would be difficult or impossible to detect after autologous transplantation where myelodysplastic syndromes are relatively common [77]. We have administered G-CSF to over 60 normal individuals to facilitate the collection of granulocytes or PBSC with the longest follow-up being four years [8, 11, 25]. These donors have not had routine follow-up examinations beyond the immediate period of PBSC or granulocyte harvests but there have been no known instances of hematologic problems. Although there is genuine concern about the long-term effects of administering recombinant G-CSF to normal individuals, the existence and frequency of such effects will not be confidently known until very large numbers of donors are evaluated over a long period of time. It is relevant to reflect that it took many donations and many years for the risks of marrow harvesting to be assessed. Presently, it is difficult to determine whether these unknown risks of G-CSF administration are outweighed by potential benefits to the donor.

In addition to the unknown long-term effects of G-CSF, donors of PBSC are subjected to one or more apheresis procedures which are generally considered to be low-risk for morbidity and mortality. However, one donor with a history of cardiac disease who was deemed to be at high risk for anesthetic complications developed a myocardial infarction after the first apheresis procedure for PBSC harvest [25]. Some donors with inadequate peripheral veins may require the placement of large bore double lumen catheters for vascular access which carries the risk of pneumothorax, bleeding and thrombophlebitis. In a series of 53 normal donors undergoing apheresis for PBSC collections at the Fred Hutchinson Cancer Research Center (FHCRC), 12 required placement of a large bore double lumen subclavian or jugular catheter while the remainder were able to undergo the procedure with a peripheral vein to vein technique. Less invasive venous access techniques in individuals with inadequate peripheral veins are needed to decrease morbidity related to PBSC collections.

The advantages to the donor of PBSC include avoidance of general anesthesia and other complications of marrow harvesting. In a review of 1,549 marrow harvests at the FHCRC, 27% had “significant” complications including 3% which were considered major (more than five units of blood administered, more than 21 days of hip pain requiring hospitalization or severe hypotension) and 0.4%

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which were considered life threatening complications (cardiac arrest, severe hypotension, septicemia and osteomyelitis) [78]. Elsewhere there have been two unreported post-operative deaths following marrow harvest, both occurring in elderly donors. Thus, any consideration of the risks to normal individuals from G-CSF administration and PBSC harvest needs to be weighed against the relatively well-defined and significant risks of marrow harvest.

PBSC Dose for Allogeneic Transplantation

The first consideration in evaluating the dose of PBSC is to determine what benchmark should be used for judging the adequacy of harvests for allogeneic transplantation. Numerous autologous studies have not found total nucleated or mononuclear cell counts to be meaningful while the results of colony forming assays vary widely and, in any case, cannot be used for real-time decision making. We have found the number of $CD34^+$ cells infused per kg of recipient body weight to be the most reliable indicator of hematopoietic adequacy for autologous transplantation [6, 7]. In the autologous situation 2.5×10^6 $CD34^+$ cells/kg is considered by some to be a minimum cell dose for consistent prompt recovery of granulocytes and platelets. At $CD34^+$ cell doses of 2.5 to 5.0×10^6/kg, compared to doses above 5.0×10^6/kg, a significant proportion of patients receiving an autologous transplant will have a delay in platelet recovery which is exacerbated by the administration of G- or GM-CSF after PBSC infusion. This phenomenon of "lineage diversion" observed after autologous PBSC transplantation should engender caution in the routine use of post-transplant myeloid growth factors following allogeneic PBSC transplantation, as prolonged thrombocytopenia could occur. We consider a dose of 5.0×10^6/kg to be the target $CD34^+$ cell dose for consistent and prompt engraftment of autologous PBSC without the administration of a post-transplant growth factor. Therefore, this should be a minimum $CD34^+$ cell dose for attempts of allogeneic PBSC transplantation.

Table 2 shows the cellular composition of 18 marrows harvested from normal unrelated donors from multiple centers as compared to 25 unmodified allogeneic PBSC grafts. The goal in this study was to collect 15×10^6 $CD34^+$ cells/kg of recipient body weight in a maximum of two apheresis procedures for allogeneic PBSC transplantation.

Table 3 summarizes the data available from the literature including an update of the Seattle experience concerning collection of PBSC from normal donors following G-CSF administration. The data reveal an increase in the number of $CD34^+$ cells collected with increasing doses of G-CSF but variations in $CD34^+$ cell yield also could be influenced by the timing of apheresis, centrifugation characteristics or the duration of apheresis. Most investigators agree that the optimal collection days for PBSC are on the fourth and fifth day of G-CSF administration and 10-20 liters of blood are processed in most studies.

Suzue et al. found that 5 μg/kg/day of G-CSF yielded significantly more $CD34^+$ cells than 2 μg/kg/day [48] while *Dreger et al.* and *Wiesneth et al.* found that 10 μg/kg/day of G-CSF yielded significantly more $CD34^+$ cells than 5 μg/kg/day [9, 61]. A direct comparison has not been made between 10 μg/kg/day versus any higher dose of G-CSF. The data in Table 2 does not permit firm conclusions, although with one exception, the number of $CD34^+$ cells collected/kg/day was highest at the 16 μg/kg/day dose. Doses of G-CSF higher than 16 μg/kg/day for PBSC in normal individuals have not been reported but would be of interest to determine if the suggested dose-response relationship persists at higher dose levels. It will be important to determine the optimal dose and schedule of G-CSF in order to maximize collections of $CD34^+$ cells and to provide background data for future studies of combinations of growth factors.

The only other growth factor evaluated in normal individuals for PBSC harvesting has been GM-CSF, which was given to four normal donors with relatively poor collections as shown in Table 3 [60]. The same investigators evaluated the combination of G- and GM-CSF in four normal donors with collection of $CD34^+$ cell numbers similar to that achieved with G-CSF alone (Table 3). Whether or not there will be advantages to different doses and schedules of G- and GM-CSF given together remains to be determined.

Although the median $CD34^+$ cell dose collected at the FHCRC after 16 μg/kg of G-CSF was high, 4 of 53 donors yielded only 0.6, 1.49, 1.55 and 1.74×10^6 $CD34^+$ cells/kg in two to four apheresis procedures. All other donors yielded 5.0×10^6 or more $CD34^+$ cells/kg. The low cell doses collected in these

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Table 3. $CD34^+$ cell collections from normal donors

Reference	G-CSF dose µ/kg	No. Donors $n = 157$	No. Apheresis/Donor	Total CD34 × 10^6/kg[b]	No. $CD34^+$ cells × 10^6/kg/per apheresis
[53]	2[a]	5	2	3.0 (2.1-3.4)	1.5 (1.0-1.7)
[48]	2	4	1	1.1	1.1
[51]	3	1	5	6.4	1.3
[48]	5	5	1	1.8	1.8
[66]	5	1	3	3.0	1.0
[73]	5	1	4	4.9	1.3
[57, 72]	6 (4.4-7.5)	14	1-2	4.5 (3.4-7.3)	4.4 (1.7-2.3)
[66]	7.5	1	2	7.7	3.4
[58]	5-10	11	2	8.7 (1.7-20.5)	4.4 (0.9-10.3)
[66]	10	6	1-3	6.5 (2.2-8.1)	3.8 (0.9-6.9)
[56]	10	4	2	4.3 (2.7-13.0)	2.2 (1.4-6.5)
[54]	10	3	3	22.4 (14.1-29.0)	7.5 (4.7-9.7)
[60]	10	7	1	1.7	1.7
[27]	12	9	3-4	10.7 (7.5-22.5)	3.6 (2.5-7.5)
[50]	12	17	2-4	11.6 (5.5-19.0)	NA
[55]	16	4	2-3	8.4 (6.4-23.0)	NA
[8, 25]	16	53	1-4	11.9 (0.6-40.1)	5.6 (0.2-20.3)
[64]	16	4	2	10.8 (4.9-11.9)	5.4 (3.4-18.5)
[60]	GM-CSF[c]	4	1	0.2	0.2
[60]	G(5) + GM-CSF[c]	4	1	1.4	1.4

[a]Fixed dose of G-CSF = 125 µg/patient.
[b]Based on recipient weight.
[c]GM-CSF = Granulocyte-Macrophage Colony Stimulating Factor, 10µg/kg/day.

four individuals would, in general, be deemed inadequate for autologous engraftment. However, despite the low cell doses, successful engraftment was achieved with all four collections. Two of the four low $CD34^+$ cell doses were infused into syngeneic recipients and resulted in prompt engraftment of granulocytes and platelets. In another instance, a patient with graft failure received a $CD34^+$ cell dose of 0.6×10^6/kg which resulted in prompt recovery of granulocytes while platelets took 31 days to recover. In the fourth instance, where the dose was 1.74×10^6 $CD34^+$ cells/kg, marrow was harvested and infused after the PBSC. The bone marrow harvest in this donor yielded only 1.5×10^8 total nucleated cells/kg. This patient had recovery of granulocytes and platelets by day 20 following administration of both marrow and PBSC.

Successful allogeneic PBSC transplants have been achieved with less than 5×10^6 $CD34^+$ cells/kg in several patients but the minimum $CD34^+$ cell dose needed for rapid and complete allogeneic engraftment will remain unknown until more patients are evaluated.

The administration of methotrexate after PBSC infusion could also affect cell dose requirements. In order to achieve the benefit of earlier engraftment, larger $CD34^+$ cell doses may be required in patients receiving methotrexate than for patients receiving immunosuppressive anti-GVHD prophylaxis regimens that are not myelosuppressive.

Cryopreservation of Allogeneic PBSC

Körbling et al. and *Russell et al.* have demonstrated that cryopreserved allogeneic PBSC can be used for allografting [27, 57, 72]. The recovery of CFU-GM and $CD34^+$ cells was good after thawing and there were no apparent problems with engraftment. Cryopreservation of allogeneic PBSC provides flexibility in the scheduling of the apheresis procedures and the transplant and all the cells can be thawed and infused in one day. However, for the majority of patients receiving PBSC transplants, fresh cells can be administered without major difficulties with avoidance of cell losses and the expense of freezing and thawing.

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T Cell Depletion Studies

Aversa et al. reduced the number of CD3 cells in PBSC to 1.24×10^5/kg with good recovery of $CD34^+$ cells using a soybean agglutination and E-rosetting technique [50].

Dreger et al. compared three other approaches to T cell depletion of PBSC, CAMPATH-1 plus autologous complement, immunomagnetic $CD34^+$ selection and biotin-avidin-mediated $CD34^+$ selection [69]. They found the immunomagnetic $CD34^+$ selection technique to be the most effective with the elimination of four logs of T cells. *Suzue et al.* reported three different techniques for T cell depletion of PBSC, all of which were associated with a large loss of hematopoietic progenitor cells [48].

One theoretical advantage of $CD34^+$ cell selection over complement lysis techniques is that a second stage selection technique could be utilized to select cell populations such as $CD56^+$ cells that could be added back to the recipient.

The only technique that has been utilized for T cell depleted PBSC allografts, without the infusion of marrow, has been $CD34^+$ selection. *Link et al.* transplanted three patients from HLA-identical siblings with a median of 6.2×10^6 $CD34^+$ cells/kg processed with a biotin-avidin affinity column technique [59]. All three patients engrafted promptly and no details are available on acute GVHD. *Rozman et al.* transplanted two patients from HLA matched siblings with 5.6 and 2.7×10^6 $CD34^+$ cells/kg separated by an immunomagnetic bead technique with prompt engraftment but no details are available on acute GVHD [63]. Although these data are limited, they document the feasibility of performing T cell depleted transplants with a $CD34^+$ selection technique.

Addition of PBSC to Marrow for Allografting

HLA Matched Allografts

In an attempt to hasten engraftment and decrease transplant-related morbidity *Nemunaitis et al.* administered G-CSF to five marrow donors and collected PBSC which were infused on days 1 to 4 post-transplant [47]. These five patients appeared to have faster engraftment than would have been expected with marrow transplantation alone and there was no apparent increase in acute GVHD.

DiPersio et al. transplanted six patients from HLA matched siblings with marrow and PBSC [49]. The marrow was unmodified and the PBSC were $CD34^+$ affinity column purified prior to infusion. The authors concluded that engraftment was not more rapid than observed with marrow and that any benefit of added $CD34^+$ cells might have been obscured by the administration of methotrexate.

Link et al. transplanted five patients with unmodified marrow and $CD34^+$ selected PBSC and five patients with marrow and PBSC that were $CD34^+$ selected. They concluded that hematopoietic recovery was accelerated as compared to marrow alone without an apparent increase in acute GVHD [59].

HLA Mismatched Allografts

Resistance to allogeneic grafts is affected by cell dose, the degree of genetic disparity between host and donor [79], transfusion induced immunity in the patient [80] and by depletion of T cells from the graft [81]. Experimental animal data have indicated that increasing the number of donor hematopoietic progenitor cells in the graft improves the probability of engraftment across an allogeneic mismatched barrier [81]. There are also data from patients with aplastic anemia receiving HLA-identical transplants demonstrating a decrease in rejection and an improved survival with the infusion of high marrow cell doses [82]. The number of donor hematopoietic cells available from marrow harvesting is limited and often suboptimal. The ability to collect PBSC after the administration of G-CSF has made it possible to test the hypothesis that increasing the dose of donor hematopoietic cells will result in more consistent engraftment.

The incidence of graft rejection has exceeded 50% in recipients of T cell-depleted marrow transplants from donors incompatible for two or three antigens using a soybean-lectin technique [83]. Using the same technique for T cell depletion not only of marrow but also of PBSC collected after G-CSF, *Aversa et al.* have reported engraftment in 16/17 patients transplanted from HLA-haploidentical donors [50]. It is also remarkable to note that despite infusion of up to 620,000 T cells/kg only one patient developed acute GVHD. However, survival was only 25% at six months, with deaths occurring predominantly from toxicity related to the very intense conditioning regimen that included cyclophosphamide, thiotepa, anti-thymocyte

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globulin and single dose TBI given at a high dose rate. Results of this study suggest that the addition of T cell-depleted PBSC assisted the establishment of allogeneic engraftment. However, T cell depletion of both marrow and PBSC requires a complicated, labor-intensive and error-prone laboratory separation procedure that may well be simplified in the future by $CD34^+$ selection. Based on the emerging data from HLA matched transplants with allogeneic PBSC it should be possible to omit the marrow harvest in future studies of T cell depletion and still infuse large quantities of $CD34^+$ cells.

Allogeneic PBSC Transplants Using PBSC Alone

Engraftment

Table 4 summarizes the engraftment data from 79 allogeneic PBSC transplants, performed without marrow, from HLA matched siblings. Most patients had rapid engraftment of both granulocytes and platelets and platelet engraftment seemed to be more rapid than historically observed with marrow. Whether the problems of platelet engraftment observed in a significant minority of allogeneic marrow recipients can be eliminated by the use of PBSC remains to be determined. Moreover, not enough patients have been evaluated to determine the early and late graft failure rate following allogeneic PBSC transplantation.

The role of post-transplant growth factors in allogeneic PBSC transplants has yet to be determined. We have not routinely administered growth factors following allogeneic PBSC transplantation because of the marginal benefit on granulocyte recovery observed following autologous PBSC transplantation and the potential detrimental effect on platelet recovery [6, 7].

In the Seattle series of allogeneic PBSC transplants, 6 of 19 patients received methotrexate as part of their prophylaxis for GVHD and there was a two to three day delay in recovery of granulocytes and platelets for these patients as compared to the 13 patients not receiving methotrexate. In patients with informative cytogenetic or molecular markers, chimerism was demonstrated which was not obviously different than that observed following marrow transplantation. Although the data are limited, with the longest follow-up being two years, there have been no late graft failures following primary transplantation, suggesting that PBSC are capable of long-term engraftment.

Graft-Versus-Host Disease

Table 5 summarizes the incidence and severity of acute and chronic GVHD in 73 evaluable recipients of HLA matched allogeneic PBSC transplants. Most patients received cyclosporine with either methotrexate or prednisone for prophylaxis of GVHD. Approximately half of the 79 patients developed grade 2 or greater acute GVHD and 17% developed grades 3-4 acute GVHD while 56% of 23 evaluable patients developed some form of chronic GVHD.

From these data it can be concluded that the incidence and severity of acute GVHD was not remarkably different from that expected after infusion of marrow. However, the incidence of chronic GVHD cannot yet be determined due to the small number and heterogeneity of the

Table 4. Engraftment after allogeneic PBSC

Reference	$n = 79$	Median Day ANC $> 0.5 \times 10^9$/l	Median Day Platelets $> 20 \times 10^9$/l
[25]	19	13 (9-22)	10 (7-42)
[27]	16	9 (8-10)	15 (8-84+)
[57, 72]	11	17 (11-26)	15 (12-109)
[58]	11	15	13
[66]	8	15 (12-23)	19 (11-39)
[56]	4	14 (9-18)	45 (13-60)
[54]	3	11 (10-12)	14 (10-18)
[75]	3	14, 20, NA	63, 50, 16
[63]	2	21,12	41,12
[64]	2	12,17	13,18

ANC = Absolute Neutrophil Count
NA = Not Available

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Table 5. GVHD After HLA-matched allogeneic PBSC transplantation

Reference	No.	Acute GVHD		Chronic GVHD all grades
		≥2	≥3	
[25]	19	10/19	4/19	7/9
[27]	16	47%	NA	5/8
[58]	10	4/10	0/10	NA
[57, 72]	8	4/8	1/8	1/6
[66]	8	2/8	1/8	NA
[56]	4	2/4	1/4	NA
[54]	3	1/3	0/3	NA
[75]	3	2/2	2/2	NA
[64]	2	0/2	0/2	NA
Total	73	25/56 (45%)	9/56 (16%)	13/23 (57%)

GVHD = Graft-Versus-Host-Disease
NA = Not Available

patients evaluated. However, if the incidence of chronic GVHD is higher following allogeneic PBSC transplantation, this would be comparable with previous experience of adding buffy coat cells to marrow in patients with aplastic anemia receiving allogeneic marrow transplants from HLA matched siblings where an increase in chronic but not acute GVHD was observed [30].

Outcome and Survival

The majority of patients given allogeneic PBSC have had advanced and often refractory hematologic malignancies, making evaluation of outcome difficult. In the Seattle series of 19 evaluable patients receiving first transplants, six patients died between days 18 and 131 of transplant-related causes, five have relapsed and eight are alive and in remission from 101 to 408 days post-transplant. Historically, this group of patients would have an approximate 10% to 20% chance of long-term disease-free survival following allogeneic marrow transplantation. It is obviously too early to predict outcome in this small group of patients.

Allogeneic Transplantation using PBSC From Mismatched Donors

Russell and colleagues have transplanted three patients with unmodified PBSC from siblings who were mismatched for 1 HLA antigen and one patient who was mismatched for 2 HLA antigens [57, 72]. One patient died on day 27 of veno-occlusive disease (VOD) with granulocyte recovery but still platelet transfusion dependent, one had prompt recovery of granulocytes and platelets, developed grade 2 acute GVHD and is alive ten months after transplantation with chronic GVHD. The third patient engrafted promptly, developed grade 2 acute GVHD and is alive eight months after transplantation with chronic GVHD. The patient receiving PBSC from a 2 antigen mismatched donor died on day 30 without engraftment. However, this patient received the lowest cell dose of the patients reported by *Russell*, 2.6×10^6 $CD34^+$ cells/kg [57, 72]. *Bacigalupo et al.* reported a successful allogeneic unmodified PBSC transplant from a donor who was DR mismatched with the recipient developing only transient acute GVHD [58].

PBSC Transplantation for Relapse after Marrow Transplantation

Körbling et al. reported four patients who underwent high dose therapy and allogeneic PBSC transplants from the same donor following relapse after an allogeneic marrow transplant [27]. In three patients acute GVHD was less than observed after the original marrow transplant and in one case it was more severe. In one of the patients the remission following the PBSC transplant is eight months longer than the remission occurring after the first transplant. *Russell et al.* reported second transplants in five patients using PBSC from the original marrow donor [57, 72]. Two patients died early of veno-occlusive disease of the liver, one died of relapse and two survive disease-free at 303 and 324 days after the second transplant. *Martinez et al.* have reported a

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successful second transplant utilizing allogeneic PBSC in a patient with AML who had relapsed 21 months after an allogeneic marrow transplant and is alive and well seven months after the second transplant [73]. We have performed a second transplant utilizing allogeneic PBSC in a patient with acute myeloid leukemia (AML) who relapsed after a T cell depleted marrow graft. He had successful engraftment without acute GVHD but died with pulmonary toxicity on day 80.

PBSC Infusion for Graft Failure

A small but significant fraction of patients have poor graft function or graft failure, usually of unknown etiology, after allogeneic marrow transplantation despite the presence of detectible donor cells in the marrow and peripheral blood. Previous studies of infusing additional unmodified marrow have been disappointing with no improvement in the aplasia and in many patients, resulting in increased acute GVHD [84]. *Dreger et al.* reported recovery of blood counts following the infusion of unmodified PBSC after two unsuccessful attempts with marrow from the same HLA-identical donor [13]. Recently *Molina et al.* reported two cases of graft failure which also were reversed with PBSC from the original HLA-identical sibling donor [68]. In both instances donor cells were detected in the marrow of the recipients prior to the infusion of PBSC. Both patients responded and have normal blood counts 280 and 324 days after the infusion of PBSC. *Kook et al.* reported the correction of graft failure, associated with the persistence of donor cells, in a patient with aplastic anemia who had received an HLA-identical marrow graft [85]. This patient received anti-thymocyte globulin and PBSC from the original donor. These results achieved in four patients with graft failure of unknown etiology after HLA-identical sibling marrow transplants are of major interest. If the findings are reproducible, the use of PBSC would help solve a major problem, as second marrow harvests within one to two months of the first harvest produce very low cell yields, cause major discomfort and are rarely successful [84].

PBSC Infusions for Graft Rejection

Immunologic graft rejection, defined as graft failure without the persistence of donor cells in marrow and blood, is difficult to treat. Further immunosuppression is probably required for successful engraftment. In the past, most patients died of complications from the toxicities of the reconditioning regimens unless the second transplant was performed for late graft failure. We have recently reported engraftment following second marrow infusions in 4/7 patients conditioned with high dose methylprednisolone and an anti-CD3 specific murine monoclonal antibody followed by cyclosporine. Two patients died of acute GVHD and two survive with normal hematopoietic function beyond two years from the second marrow infusion [86]. Nine similar patients with graft rejection have received methylprednisolone and the same anti-CD3 specific murine monoclonal antibody followed by allogeneic PBSC from the original marrow donor. Engraftment occurred in 6/9 patients, four of whom survive with good grafts more than 100 days after allogeneic PBSC infusion. Acute GVHD, grade 2-3, occurred in 2/6 patients with engraftment despite the infusion of an average of 6.5×10^9/kg $CD3^+$ T cells obtained in all but one case from HLA-mismatched or unrelated donors. It remains to be determined if there are advantages to using PBSC rather than marrow in this situation. However, all the donors who have donated marrow and PBSC have voiced a preference for the PBSC harvest if given a choice.

Allogeneic PBSC Infusions for Relapse

Lymphocyte infusions from the original marrow donor have been used as immunotherapy for post-transplant relapses with some success, especially in patients with chronic myeloid leukemia (CML) [87]. However, a significant fraction of patients with CML who receive infusions of buffy coat cells from their marrow donor develop pancytopenia. Allogeneic PBSC collected after G-CSF potentially provide lymphocytes for a graft-versus-leukemia effect but also provide hematopoietic precursors which should prevent aplasia. *Maiolino* has given allogeneic PBSC without prior ablative therapy to two patients who relapsed after an allogeneic marrow transplant [55]. One patient with CML with a cytogenetic relapse achieved a remission without aplasia. The second patient had ALL in remission and was not evaluable for response. Grade 2 acute GVHD occurred in one of the two patients. It is likely that G-CSF mobilized PBSC will replace buffy coat infusions in the near future because of the added advantage of infusing functional hematopoietic cells.

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Current and Future Studies

Animal and laboratory studies are being carried out to determine the qualitative differences between marrow and PBSC. The immediate goal for improvements in procurement of PBSC is to obtain enough cells for engraftment in one to two apheresis procedures. This may be difficult if T cell depletion techniques are utilized due to cell losses from the procedure. Peripheral blood stem cell yields could certainly be improved by enhancing the centrifugation efficiency. However, it will be difficult to improve centrifugation techniques without the ability to perform prompt, reliable and reproducible $CD34^+$ measurements. It will also be important to improve vascular access techniques for individuals with inadequate peripheral veins as current techniques are associated with significant complications. Further long-term evaluation of normal donors who have received growth factors for PBSC mobilization will help answer questions about the safety of this approach. An important future goal will be to develop safe and effective growth factor regimens that will facilitate collection of adequate numbers of PBSC from one to two units of blood with avoidance of apheresis altogether. A randomized controlled trial of allogeneic marrow versus PBSC is currently being carried out by the European Bone Marrow Transplant Registry, which should answer questions related to engraftment, acute and chronic GVHD, relapse and survival differences.

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