Allogeneic Blood Stem Cell Transplantation for Refractory Leukemia and Lymphoma: Potential Advantage of Blood Over Marrow Allografts

By M. Körbling, D. Przepiorka, Y.O. Huh, H. Engel, K. van Besien, S. Giralt, B. Andersson, H.D. Kleine, D. Seong, A.B. Deisseroth, M. Andreeff, and R. Champlin

Peripheral blood stem cells (PBSCs) have been used rarely for allogeneic transplantation because of concerns regarding graft failure and graft-versus-host disease (GVHD). We evaluated the results of allogeneic PBSC transplantation (allo-PBSCT) in 9 patients with refractory leukemia or lymphoma receiving myeloablative therapy followed by allo-PBSCT from an HLA-identical sibling donor. Three patients had relapsed 11 to 21 months after allogeneic bone marrow transplantation (allo-BMT) and underwent allo-PBSCT using the same donor. Six patients received PBSCs as their initial allogeneic transplant. Filgrastim-mobilized PBSCs were collected from the donors in 3 to 4 aphereses and cryopreserved. The apheresis collections contained a median nucleated cell count of 16.5 × 10^8/kg (range, 10.8 to 28.7 × 10^8), 10.7 × 10^6 $CD34^+$ cells/kg (range, 7.5 to 22.5 × 10^6), and 300.0 × 10^6 $CD3^+$ cells/kg (range, 127.8 to 1,523.2 × 10^6). The median recovery of $CD34^+$ progenitor cells after freezing, thawing, and washing was 106.4% (range, 36.7% to 132.0%). All patients received filgrastim posttransplant through engraftment, and cyclosporine and methylprednisolone were used for GVHD prophylaxis. Neutrophil recovery to greater than 0.5 × 10^9/L and greater than 1.0 × 10^9/L occurred at a median of 9 (range, 8 to 10) and 9 days (range, 8 to 11) posttransplant, respectively, which was similar to historical controls after allo-BMT and granulocyte colony-stimulating factor therapy. Platelets recovered to greater than 20 × 10^9/L and greater than 50 × 10^9/L at a median of 12 (range, 8 to 25) and 15 days (range, 11 to 59), respectively, which was significantly more rapid than for the controls ($P < .01$). Donor cell engraftment was documented by cytogenetics, fluorescence in situ hybridization, and/or restriction fragment length polymorphisms with longest follow-up of 283+ days. Three patients developed grade 2 acute GVHD involving only the skin. Three of five evaluable patients show limited chronic GVHD. Cryopreserved, filgrastim-stimulated allogeneic PBSCs may be a suitable alternative to allogeneic marrow for transplantation with the advantage of more rapid platelet recovery. Acute GVHD was minimal despite the infusion of 1 log more CD3 cells than with marrow allografts. Further studies are required to assess long-term risks of chronic GVHD.

TRANSPLANTATION of autologous peripheral blood stem cells (PBSCs) is a well-established method to reconstitute hematopoiesis in patients receiving a myeloablative preparative regimen.[1,2] Allogeneic PBSCs have been used rarely as the sole support for hematopoietic reconstitution after myeloablative treatment because of questions regarding durability of engraftment and risk of graft-versus-host disease (GVHD).[3,4] Treatment of normal donors using filgrastim has been reported as safe and effective for collecting granulocytes for transfusion[5] and for mobilization of sufficient hematopoietic progenitors for transplantation.[6,7] Such PBSCs have been used successfully for treatment of graft failure in the absence of immunologic rejection,[8] for syngeneic transplantation,[9] and to hasten engraftment after allogeneic marrow transplantation.[10] We report here a series of nine patients with refractory leukemia or lymphoma transplanted with allogeneic PBSCs as the sole source of reconstituting cells with the focus on (1) yield of apheresis-derived hematopoietic progenitor cells and lymphoid cells from HLA-identical sibling donors, (2) cell recovery after freezing and thawing, (3) the kinetics of hematopoietic reconstitution, (4) the rate of acute GVHD, and (5) the durability of the blood stem cell allograft in comparison to historical controls receiving marrow transplants.

DONORS, PATIENTS, AND METHODS

Blood stem cell donors. The blood stem cell donors were HLA-identical siblings. Donor hematopoietic progenitor cells were mobilized into the peripheral blood using filgrastim at a dose of 6 μg/kg subcutaneously (SC) every 12 hours administered through completion of stem cell collection.

Blood stem cell collection, cryopreservation, and thawing. Apheresis was performed daily for 3 days (8 donors) or 4 days (1 donor) beginning on day 4 of filgrastim administration. PBSCs were collected using a Cobe Spectra blood cell separator (Cobe BCT, Inc, Lakewood, CO), and the total blood volume processed per apheresis was between two and three times the donor's blood volume. All apheresis products were frozen at a controlled rate (Model 1010; Cryomed, Marietta, OH) with 5% dimethylsulfoxide (DMSO; Cryoserv, Research Industries Corp, Salt Lake City, UT) in 100 mL volumes in two to four freezing bags (Stericon, Inc, Chicago, IL) per apheresis. On day 0, the frozen apheresis products were thawed, pooled, and washed free of DMSO using the Fenwal CS 3000 blood cell separator (Baxter Healthcare Corp, Deerfield, IL) and infused.

Immunophenotyping. The apheresis products, control marrow harvests, and the recipients' peripheral blood samples posttransplant were analyzed by two- or three-color flow cytometry. Cells (1 × 10^6) of each sample were resuspended in 100 μL of phosphate-buffered saline (PBS) containing 1% bovine serum albumin and stained with monoclonal antibodies (Becton Dickinson, Mountain View, CA) for CD34-FITC (clone 8G12), CD38-PE (clone HB7) and HLA-DR-PerCP (clone L243) at 10 μL each. Isotype controls combined with specific antibodies were used for setting amplification

From the Sections of Bone Marrow Transplantation, Apheresis, Transfusion Medicine, and Experimental Hematology, U.T.M.D. Anderson Cancer Center, Houston, TX.

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Address reprint requests to M. Körbling, MD, U.T.M.D. Anderson Cancer Center, Department of Hematology, 1515 Holcombe Blvd, Box 068, Houston, TX 77030.

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Table 1. Patient Characteristics, Engraftment, and Chimerism

Patient No.	Diagnosis	Patient Age/Gender	Donor Age/ Gender	Preparative Regimen	Days to Neutrophils		Days to Platelets		Latest Chimerism/MRD Study	
					>0.5 × 10^6/L	>1.0 × 10^6/L	>20 × 10^9/L	>50 × 10^9/L	Cytogenetics	RFLP
1	AML	24/F	28/M	CY/TBI	10	10	11	11	20/20 46,XY [283]*	Donor [173]
2	AML	46/M	49/M	TBC	9	9	12	15	20/20 46,XY [132]	Donor [132]
3	Hodgkin's	24/M	22/F	CBV	9	9	51	59	20/20 46,XX [102]	Donor [30]
4	Lymphoma	36/F	28/M	TBC	8	8	9	13	—	Donor [55]
5	CML	51/M	46/M	CY/TBI	9	9	14	14	20/20 46,XY [83]	Donor [84]
6	Lymphoma	39/M	29/F	TBC	9	9	10	17	21/21 46,XX [28]	Donor [28]
7	Lymphoma	58/F	50/F	TBC	9	10	27	28	—	—
8	Lymphoma	39/M	41/M	TBC	9	9	9	9	—	—
9	AML	38/M	40/M	CY/TBI	10	11	14	23	—	—
Control group					9 (8-16) NS	10 (8-19) NS	19 (11-100+) $P < .01$	27 (13-100+) $P < .01$		

Abbreviations: CY/TBI, cyclophosphamide, total body irradiation; TBC, Thiotepa, busulfan, CY; CBV, CY, BCNU, etoposide; NS, not significant.
* Patients no. 1, 2, and 5 had clonal cytogenetic abnormalities while in relapse. Numbers in brackets refer to transplant day of latest study.

and compensation of the flow cytometer. The following combinations of control settings were used: (IgG1-FITC, IgG1-PE, IgG2a-PerCP), (CD34-FITC, IgG1-PE, IgG2a-PerCP), (IgG1-FITC, CD38-PE, IgG2a-PerCP), and (IgG1-FITC, IgG1-PE, HLA-DR-PerCP). Cells were analyzed on a FACSCAN (Becton Dickinson, Palo Alto, CA) equipped with a 15 mW Argon ion laser tuned emitting at 488 nm. FITC-immunofluorescence (FL 1), PE-immunofluorescence (FL 2), and PerCP-immunofluorescence (FL 3) were measured at 530, 585, and 650 nm, respectively. All fluorescence parameters were amplified logarithmically. The scatter parameters (90° side and forward scatter) were amplified linearly. A Hewlett-Packard 300 computer system (Hewlett-Packard, Roseville, CA) was used for processing of list mode data. $CD34^+$ cells were quantified as a two-step procedure. (1) From the analysis of forward and 90° side scatter (10,000 events), a gate was established to include all lymphocytes and mononuclear cells, excluding granulocytes. Controls were set on the basis of those gated cells. (2) A "life-gate" was set on high $CD34^+$ immunofluorescence and low side-scatter. One thousand "life-gate" events were accumulated. The ratio "life-gate" events to total events acquired determined the percentage of $CD34^+$ cells among the total cell population. $CD34^+$ subsets were evaluated according to quadrant distribution. Immunophenotyping of lymphocytes was done as described above but without "life gating" or by two-color flow cytometric analysis.[11]

Allogeneic PBSC transplant (allo-PBSCT) recipients. Eleven patients underwent allo-PBSCT from October 1, 1993 to July 31, 1994. Two patients are excluded from this report; one died on day 4 from viral pneumonia and the second died on day 6 from cardiac complications and infection. Characteristics of the remaining nine patients are shown in Table 1. The median age was 39 years (range, 24 to 58 years). All were refractory to conventional chemotherapy and salvage regimens. Patients no. 1, 5, and 9 were in relapse 11 to 21 months after allogeneic bone marrow transplantation (allo-BMT) using a busulfan-based preparative regimen.

Treatment and supportive care. All patients received a myeloablative regimen as indicated in Table 1.[12-15] Cyclosporine and methylprednisolone were used for GVHD prophylaxis. Cyclosporine was initiated on day −2 at 3 mg/kg/day intravenously (IV) by continuous infusion, and the dose was adjusted to maintain a whole blood radioimmunoassay (RIA) level of 200 to 300 ng/mL. Methylprednisolone was administered at 0.5 mg/kg IV twice daily on days 5 to 28, with the dosage tapering down slowly thereafter. The diagnosis and grading of acute GVHD were made on clinical evaluation with histologic confirmation.[16] Filgrastim (5 μg/kg SC) was administered daily from day 1 through engraftment.

The patients were hospitalized in laminar airflow rooms. Infection prophylaxis during the peritransplant period consisted of nonabsorbable antibiotics orally, 1 g vancomycin IV daily, 200 mg fluconazole IV twice daily, and 5 mg/kg acyclovir IV every 8 or 12 hours. All patients received broad spectrum antibiotics for neutropenic fever, hyperalimentation when needed, and irradiated blood products per standard routine. IV Ig (500 mg/kg) was administered weekly through day 100 and monthly thereafter through 1 year. Once engrafted, the patients also received twice-weekly trimethoprim/sulfamethoxazole orally or pentamadine by inhalation every 3 weeks; cytomegalovirus (CMV)-seropositive patients received prophylactic ganciclovir 5 times per week.

Documentation of engraftment and remission. The day of granulocyte engraftment was defined as the first of 3 consecutive days with a granulocyte count exceeding 0.5 × 10^9/L. The day of platelet engraftment was the day the platelet count exceeded 20 × 10^9/L without a platelet transfusion for at least 7 days thereafter. Donor cells in the marrow or peripheral blood were identified by DNA restriction fragment length polymorphisms (RFLP) at the AY-29 or YNH24 locus.[17] Cytogenetic analysis was performed on unstimulated marrow using conventional methods. Fluorescence in situ hybridization (FISH) for chromosomes X, Y, and 7 using centromeric probes (Imagenetics, Framingham, MA) was performed as described previously.[18] Using FISH for chromosome 7, normal peripheral blood buffy coat cells had 1.17% ± 0.67% (mean ± SD) with 1 signal.

Statistical analysis of engraftment. For comparison of outcome, data were obtained for a historical control group composed of patients with advanced hematologic malignancies participating in a study of thiotepa, busulfan, and cyclophosphamide for allogeneic marrow transplantation using filgrastim to enhance engraftment, and cyclosporine and methylprednisolone for GVHD prophylaxis.[14] This is the only institutional protocol using this hematopoietic growth factor and GVHD prophylaxis regimen. The group included 7 men and 18 women (median age, 33 years; range, 16 to 52 years) with HLA-identical marrow donors. Two patients underwent second transplants more than 1 year after the first transplant. All patients were evaluable for engraftment and GVHD. A generalized Wilcoxon test was used to compare neutrophil and platelet reconstitution data after allo-PBSCT with those of the historical allo-BMT group. Only a proportion of the patients had marrow immunophenotyping; all such data available are reported.

RESULTS

Immunophenotypic characterization of the apheresis collections. The total apheresis collections had a mean num-

Table 2. Characteristics of Apheresis Collections

Donor	Total Nucleated Cells		$CD34^+$		$CD3^+$	$CD4^+$	$CD8^+$	$CD19^+$	$CD56^+3^-$
	Collected	Postthaw	Collected	Postthaw					
1	28.7	19.4	22.4	24.0	127.8	193.8	41.0	26.1	91.5
2	11.9	10.3	7.5	10.0	211.1	153.7	47.3	44.2	37.3
3	16.5	8.5	20.1	25.0	309.9	236.6	131.9	38.7	41.1
4	16.4	7.8	22.5	26.0	190.2	313.1	24.2	57.3	91.4
5	19.4	5.0	10.7	12.8	277.5	171.1	104.6	77.5	20.7
6	14.9	9.7	7.6	5.0	506.9	286.9	240.3	83.5	77.9
7	28.6	14.8	7.9	2.9	1,523.2	960.0	465.9	121.4	69.5
8	18.4	14.2	10.9	11.6	299.5	189.7	145.6	60.9	82.3
9	10.8	7.4	9.1	5.3	426.5	273.7	189.3	97.2	67.1
Mean	18.4	10.8	13.1	13.6	430.2	308.7	153.5	67.4	64.3
(SE)	(2.1)	(1.5)	(2.2)	(3.0)	(142.0)	(83.4)	(44.8)	(10.1)	(8.5)
Control group*	3.2		3.4		27.2	20.4	6.4	7.4	3.2
	(0.2)		(0.6)		(5.4)	(4.1)	(1.7)	(2.0)	(0.7)

Values are expressed as 10^{-8}/kg recipient's weight for total nucleated cells and 10^{-6}/kg recipient's weight for the other subsets listed.
* Mean (SE) of seven collections from control group.

ber of total nucleated cells (TNC) of 18.4×10^8/kg recipient's weight; a mean TNC of 10.8×10^8/kg (60%) were recovered after thawing and washing (Table 2). This was approximately threefold more cells than used for marrow allografts. The $CD34^+$ subset comprised 0.3% to 1.4% (mean, 1.1%) of the cells collected. The mean number of $CD34^+$ cells collected was 13.1×10^6/kg recipient's weight, and few $CD34^+$ cells were lost after washing (Table 2). The total number of $CD34^+$ cells infused was increased fourfold in comparison to the normal marrow harvests.

The mean number of lymphocytes collected was 5.8×10^8/kg recipient's weight. The number of lymphocytes of particular subsets in the apheresis collections were 1.0 to 1.4 logs higher than in normal marrow harvests (Table 2). Data were available from two patients to determine the recovery of cells of each lymphocyte subset after thawing and washing; there was no evidence of loss of cells from a particular subset (data not shown).

Engraftment. Neutrophil engraftment was rapid in all patients with recovery at a median of 9 days (Table 1). However, this was similar to recovery in the historical control group with similar growth factor support and immunosuppressive therapy after allo-BMT. Platelet recovery was also rapid after allo-PBSCT. The median time to platelet counts exceeding 20×10^9/L (12 days) or 50×10^9/L (15 days) was significantly shorter than for the historical control group ($P < .01$ for both comparisons; Table 2). Engraftment was stable. No patient has developed late graft failure while in remission.

Circulating hematopoietic progenitors were serially assessed by flow cytometry posttransplant. The numbers of $CD34^+$ cells and more primitive subsets of $CD34^+$ cells peaked early after allo-PBSCT, between days 10 and 13, while the patients were receiving filgrastim. The number of circulating hematopoietic progenitors decreased after filgrastim was discontinued but recovered over 50 to 56 days posttransplant (Fig 1).

Chimerism and minimal residual disease. All evaluable patients had complete donor chimerism in marrow samples as determined by cytogenetics and/or RFLP (Table 1). Patient no. 1 also had 85 of 85 interphase cells positive for X and Y chromosomes by FISH at day 60.

In Patient no. 1, leukemia cell metaphases identified by the t(16;17) were not found posttransplant (Table 1). However, in Patient no. 2, despite a marrow in morphologic remission, cytogenetic abnormalities consistent with the original leukemia were detected in a small number of cells posttransplant. During first remission, the karyotype was 46,XY. At the time of initial relapse, the karyotype was 45,XY,inv(3),−7, but no −7 was present after reinduction was attempted with conventional chemotherapy pretransplant. At 3 weeks posttransplant, 1 of 30 metaphases had the inv(3). At 2, 4, 8, and 19 weeks posttransplant, 3 of 500 (0.6%), 5 of 500 (1.0%), 4 of 500 (0.8%), and 4 of 500 (0.8%) interphase cells, respectively, had a single chromosome 7 by FISH. These levels are within background range for this probe (mean, 1.17% ± 0.67% with −7 for normal peripheral blood buffy coat cells). Patient no. 5 had no evidence of the Philadelphia chromosome as late as day 83 posttransplant, and the bcr gene was germline by Southern blot analysis.

GVHD. Three of the nine patients developed isolated stage 3 acute GVHD of the skin (Table 3); two responded rapidly to treatment with high-dose methylprednisolone and one resolved with topical therapy. Patient no. 1 had no acute GHVD after either the first or second transplant, patient no. 5 had grade 2 cutaneous and visceral GVHD after his first transplant and only skin involvement after the second transplant, and patient no. 9 had skin involvement after the first transplant and no GVHD after the second transplant to date. None of the allo-PBSCT recipients required ATG. For the control group, the rate of grades 2 to 4 acute GVHD was 70%, and 94% of the patients who developed acute GVHD did so within 1 month posttransplant.

Five patients have been observed for at least 100 days posttransplant and three have developed chronic GVHD (Table 3). Two have localized involvement and are on no treatment or topical therapy alone.

Outcome. One patient died of parainfluenza pneumonia and sepsis 82 days after allo-PBSCT. No evidence of

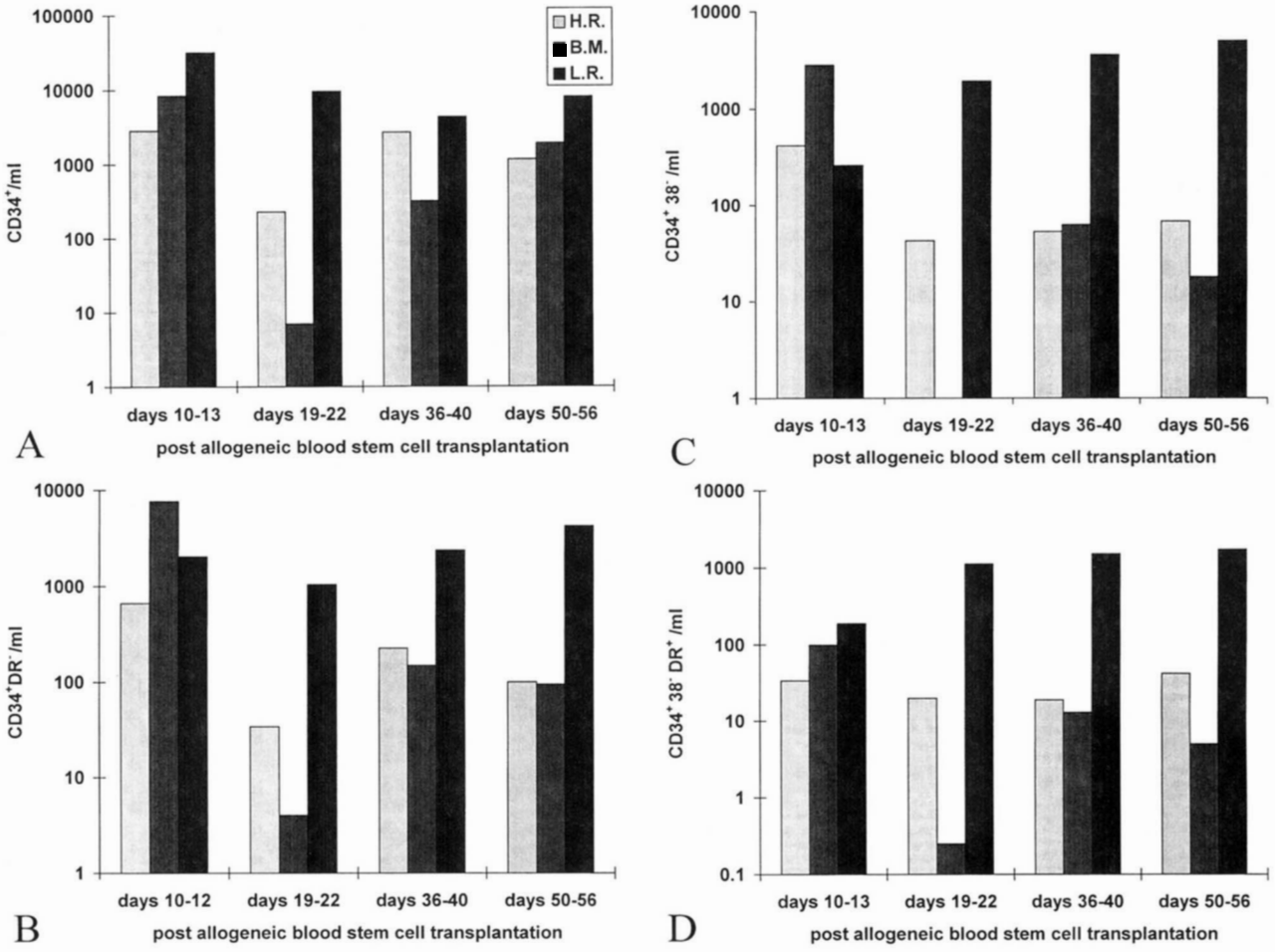

Fig 1. Recovery of hematopoietic progenitor cells after allo-PBSCT in patients no. 1, 2, and 6. (A) Recovery of $CD34^+$ cells/mL. (B) Recovery of $CD34^+DR^-$ cells/mL. (C) Recovery of $CD34^+CD38^-$ cells/mL. (D) Recovery of $CD34^+CD38^-DR^+$ cells/mL.

lymphoma was found at autopsy. The remainder of the patients are alive 24+ to 326+ days posttransplant. One patient with lymphoma achieved a partial remission, and the remaining patients are all in complete remission. For patient no. 1 the remission duration after allo-PBSCT has been 4 months longer than that after allo-BMT.

DISCUSSION

The long-term reconstitutive capability of blood-derived hematopoietic stem cells has not yet been proven after allogeneic transplantation in humans. Using sex-mismatched murine transplants and a molecular probe for Y-chromosome–specific DNA sequences, Molineux et al[19] showed

Table 3. GVHD and Outcome

Patient No.	Maximum Acute GVHD					Chronic GVHD		Survival
	Skin	Liver	Gut	Overall	Day Onset	Grade	Day Onset	
1	0	0	0	0	—	Limited	160	Day 326+ CR
2	3	0	0	2	8	Limited	114	Day 208+ CR
3	0	0	0	0	—	Extensive	99	Day 123+ CR
4	0	0	0	0	—	—	—	Day 82 infection (CR)
5	3	0	0	2	44	0	—	Day 121+ CR
6	0	0	0	0	—	0	—	Day 100+ CR
7	0	0	0	0	—	—	—	Day 38+ PR
8	3	0	0	2	26	—	—	Day 31+ CR
9	0	0	0	0	—	—	—	Day 24+ CR

Abbreviations: CR, complete remission; PR, partial remission.

that granulocyte colony-stimulating factor (G-CSF)–primed blood stem cell transplants are capable of durably reconstituting the hematopoiesis. Carbonell et al[20] reported that DLA-mismatched canine blood stem cell transplantation resulted in long-term donor type engraftment for up to 3.2 years posttransplant. In this study, we report nine patients with successful lymphohematopoietic engraftment of allogeneic, HLA-identical PBSCs after myeloablative therapy. With a longest follow-up of 326+ days after allo-PBSCT, all three cell lineages of the hematopoietic system continue to function, indicating a sufficient self-renewal capacity of frozen/thawed, blood-derived stem cells. When exposed to stem cell suppressive drugs such as methotrexate or ganciclovir, the hematopoietic function after allo-PBSCT did not seem to be more susceptible than after allo-BMT.

Low levels of the $CD34^+CD38^-$ subset, which includes putative reconstituting stem cells,[21] were present and peaked in the early recovery phase after allo-PBSCT. This is consistent with a recent study demonstrating that G-CSF– and granulocyte-macrophage colony-stimulating factor (GM-CSF)–mobilized apheresis products contain $CD34^+$ Thy-1^+ and $CD38^-$ subsets.[22]

Efficient blood stem cell mobilization in normal donors using filgrastim allows apheresis collections to match or exceed a single marrow harvest in progenitor content without exposing the donor to the risks of anesthesia and a marrow harvest. Sheridan et al[23] reported a median 58-fold increase in colony-forming unit granulocyte-macrophage (CFU-GM) over pretreatment values with filgrastim at 12 μg/kg/d. The optimal cell dose and number of $CD34^+$ cells for allogeneic PBSCT is unknown. Matsunaga et al[6] suggested that an engrafting dose of PBSCs could be collected in as little as one apheresis from donors receiving filgrastim at a dose of 2.5 μg/kg for 6 successive days followed by 5 μg/kg for the following 4 days. Fritsch et al[7] also reported that, by a single leukapheresis, sufficient PBSCs can be collected from a cytokine-stimulated normal donor to ensure hematopoietic engraftment in an adult recipient. Our data show that, using filgrastim at 6 μg/kg SC twice daily, a target engraftment dose of 4×10^6 $CD34^+$ cells/kg could also be collected with 2 or less aphereses. But, even in normal donors, some variability in cytokine-mobilized and apheresis-derived stem cell yield has to be taken into consideration.

Neutrophil recovery was rapid in our patients after allogeneic PBSCT, but it did not occur any earlier than for the historical control group that did not receive methotrexate for GVHD prophylaxis after allo-BMT. In contrast, platelet recovery was more rapid in our patients after allo-PBSCT than for the allo-BMT historical control group, similar to the experience with autologous PBSCT.[23,24] Filgrastim itself does not affect platelet recovery after allogeneic or autologous BMT. However, filgrastim does increase numbers of circulating megakaryocyte progenitors in PBSC donors,[25] and this has been associated with an accelerated platelet recovery in recipients of autologous filgrastim-stimulated PBSCs.[23]

It is uncertain whether infusion of larger numbers of lymphocytes present in the PBSC allograft will improve immune reconstitution posttransplant. For autologous recipients, Roberts et al[26] reported that total T cells and $CD4^+$ cell number increased more rapidly after PBSCT than after BMT, whereas Henon et al[27] found little difference in immunologic recovery between PBSC and marrow recipients. Prolonged use of cyclosporine may obviate any advantage of PBSCT for immune reconstitution, but further studies are warranted to fully investigate this.

Whether there is a linear relationship between the number of T cells infused and the development of GVHD as postulated by Owens and Santos[28] in murine studies or whether above a certain number of T cells a plateau is reached at which MHC disparity is the major GVHD inducing factor remain to be determined. Nonetheless, despite the large numbers of T cells administered with the allo-PBSCT, GVHD was mild or nonexistant in our patients. However, the observed presence of GVHD in target organs such as skin is in agreement with data reported by Sanders et al,[29] who noticed GVHD after second transplant even if not present after the first transplant. In our experience of two evaluable patients who underwent a second allo-PBSCT, acute GVHD was less than after first allo-BMT or nonexistent. Eckardt et al[30] have also noted that cryopreservation of allogeneic marrow reduced the risk of acute GVHD, possibly through selective depletion of or induction of anergy in GVHD-inducing cells. How cryopreservation affects the alloreactivity of PBSCs needs to be investigated further.

The PBSC collections also contained a high number of $CD3^+CD4^-CD8^-$ (data not shown) and $CD3^-CD56^+$ subsets that may include the natural suppressor cells thought to be responsible for inhibition of GVHD effect early posttransplant,[31,32] but the numbers or ratios of the lymphocyte subsets for optimal activity has not been determined.

It is noteworthy in our study that, at the time of writing, the duration of post allo-PBSCT remission exceeds the prior post allo-BMT remission in patient no. 1 by 4 months, leading to speculation regarding a possible additional graft-versus-leukemia (GVL) effect after allo-PBSCT. T lymphocytes present in the allogeneic graft confer a GVL effect, as evidenced by the increased relapse rate after T-cell–depleted BMT and the induction of remission by infusion of donor leukocytes in patients relapsing posttransplant.[33] With increased numbers of T lymphocytes and natural killer cells present in PBSC collections, it remains to be determined whether these cells are able to confer an enhanced GVL effect.

In conclusion, a number of considerations might favor the circulating blood as a stem cell source for allografting: (1) shorter duration of posttransplant aplasia, (2) faster hematopoietic and/or immune-reconstitution, and (3) a potentially more pronounced GVL effect. In addition, general anesthesia is not necessary for collection and the procedure results in less discomfort for the donor. This experience shows that allo-PBSCT is feasible and warrants further clinical trials. With confirmation of the safety and efficacy of allo-PBSCT, this new technology has the potential to become the basis for an unrelated blood stem cell donor program that from a logistical point of view might not be very different from current apheresis donations.

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NOTE ADDED IN PROOF

As of December 15, 1994, the total number of evaluable patients with refractory leukemia, lymphoma, or MDS who received a PBSC transplant from their HLA-identical sibling donors increased to 16. The longest follow-up with proven donor chimerism (RFLP) is 463+ days, with a median follow-up of 121 days. The median time to ANC greater than 500/μL and ANC greater than 1,000/μL was 9 days (range, 8 to 10 days) and 9 days (range, 8 to 11 days), respectively, and 12 days (range, 8 to 87+ days) and 15 days (range, 8 to 87+ days) to platelets greater than 20,000/μL, and greater than 50,000/μL, respectively. Platelet recovery after allo-PBSCT was significantly faster than after allo-BMT ($P < .01$). The actuarial rate of grades 2-4 acute GVHD was 47%; 2 of 8 evaluable patients developed limited chronic GVHD and 3 of 8 evaluable patients developed clinically extensive chronic GVHD. Four patients underwent an allo-PBSCT after relapse from allo-BMT. In 3 patients, acute GVHD was less than after allo-BMT; in 1 patient, acute GVHD became more severe. In patient no. 1, the duration of post–allo-PBSCT remission exceeds the prior post–allo-BMT remission by 8 months.

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