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Transplantation of Allogeneic CD34+ Peripheral Blood Stem Cells in Patients With Advanced Hematologic Malignancy

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Sixteen patients with advanced hematologic malignancies were transplanted with HLA-identical allogeneic peripheral blood stem cells (PBSCs) that were selected for CD34+ cells by an avidin-biotin immunoadsorption technique. The median age of patients was 48 years (range, 37 to 67). Patients received 12.0 or 13.2 Gy of total body irradiation followed by 120 mg/kg of cyclophosphamide. Normal donors received 16 mg/kg of granulocyte-colony stimulating factor on days 1 to 6 followed by PBSC harvests on days 4 to 7. PBSC harvests were processed each day on a single avidin-biotin column containing an antibody to the CD34 antigen and processed cells were infused without cryopreservation daily for 4 consecutive days. Prophylaxis against graft-versus-host disease (GVHD) consisted of cyclosporine alone for 5 patients and CSA plus methotrexate for 11 patients. A median of 18.64 (6.74 to 34.97) × 10^6 CD34+ cells/kg patient body weight were collected from each donor. A median of 8.96 (2.62 to 17.34) × 10^6 CD34+ cells/kg patient body weight were recovered after avidin-biotin adsorption which represented a median CD34+ cell yield of 53% (18% to 77%) with a median purity of 62% (34% to 82%). There was a reduction in CD3+ cells from a median of 557.26 (227.73 to 677.77) × 106/kg to 0.73 × 10^6/kg (0.40 to 3.65), in CD4+ cells from 351.72 (194.47 to 520.11) × 10^6/kg to 0.40 (0.15 to 1.03) × 10^6/kg and in CD8+ cells from 169.74 (53.34 to 325.83) × 10^6/kg to 0.32 (0.12 to 2.71) × 10^6/kg representing a median 2.8 (2.19 to 3.14) log reduction in T cells. One patient died of infection on day 3 posttransplant and was unevaluable for recovery of neutrophils. The median day to recovery of 500 neutrophils/mL was 15 (8 to 26) in the remaining 15 patients. Six of 16 patients failed to achieve a platelet count of 20,000/mL before death on days 3 to 97 of transplant-related complications. The median day to achieving platelets of 20,000 mL in the remaining 10 patients was 11 (7 to 31). Eight of 16 patients (50%) died between 3 and 97 days posttransplant, 7 of transplant-related causes, and 1 of progressive disease. Grade 2-4 acute GVHD occurred in 12 out of 14 (86%) and grades 3-4 in 6 out of 14 (43%) evaluable patients. Six of 8 evaluable patients developed clinical chronic GVHD and 1 developed subclinical chronic GVHD. Bone marrow and/or peripheral blood chimerism studies in 12 evaluable patients showed 97% to 100% donor type in 11 patients with 1 patient in relapse showing 40% donor cells 60 to 90 days posttransplant. Four of 16 patients (25%) are alive and disease-free 312 to 576 days after transplant. There were no episodes of graft failure or rejection. This study shows that allogeneic transplantation using CD34+ selected PBSC results in prompt and sustained engraftment. CD34+ selection, as employed in this preliminary study, however, resulted in an apparently higher rate of acute and chronic GVHD. However, The sample size is quite small and precludes a more definitive conclusion regarding GVHD.

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RECENT STUDIES have evaluated the use of granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs) as an alternative to bone marrow (BM) as a source of allogeneic hematopoietic stem cells.[1-3] Preliminary data suggest that allogenic PBSC transplants result in more rapid engraftment without an apparent increase in acute GVHD as compared to BM, but the numbers of patients treated are too few and follow-up too short to make any conclusions concerning effects on chronic graft-versus-host disease (GVHD), relapse or survival. However, the large number of T cells in PBSC collections raises the concern that allogeneic grafts using PBSCs may be associated with more GVHD, especially if applied in settings where GVHD is of greater risk, such as in older patients or in patients receiving mismatched or unrelated transplants. The ability to mobilize and collect large quantities of PBSCs, as measured by the CD34+ cell content, offers unique opportunities for graft manipulation.

Allogeneic transplants using T-cell depleted BM are associated with a lower incidence of GVHD but an increase in the incidence of graft failure and relapse.[4,5] Although the increased incidence of graft failure is usually attributed to the low number of T cells infused, there is also a considerable loss of CD34+ cells following most methods of T-cell depletion which could contribute to graft rejection or graft failure as well as delayed rates of hematopoietic recovery.[6-8] Allogeneic PBSC grafts have contained 3 to 4 times more CD34+ cells than BM, potentially allowing for T-cell depletion techniques to be performed with retention of relatively large numbers of CD34+ cells.[9-12] Initial in vitro studies showed that CD34+ selection techniques could remove 2 to 3 logs of T cells from PBSC collections and preliminary clinical pilot studies have documented the feasibility of performing transplants with CD34+ cell enriched PBSC.[13,14] This pilot study was conducted to test whether rapid, sustained engraftment could be achieved with CD34+ selected G-CSF mobilized PBSCs from matched donors and whether CD34+ selection had any apparent impact on the development of GVHD. An older population (age >35) was chosen for this study because this population is at greater risk for developing severe complications from GVHD and the risks of CD34+ selection were better balanced by potential benefit.

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Table 1. Patient and Donor Characteristics

UPN	Diagnosis	Phase at Tx	Age	Gender		CMV Serology	
				Patient	Donor	Patient	Donor
8,887	MDS	RAEB	67	M	M	+	−
9,141	AML	3rd relapse	58	F	F	+	+
6,709	AML	2nd relapse	44	M	F	+	+
9,337	NHL	2nd relapse	43	M	M	−	−
9,426	CML	Blast crisis	58	M	F	+	+
733	AML	2nd remission	34	M	F	−	−
1,032	CML	Blast crisis	42	F	M	+	+
9,364	NHL	Refractory relapse	49	M	M	−	−
1,036	AML	Refractory relapse	45	F	M	+	+
1,039	AML	Refractory relapse	37	M	M	+	+
9,380	AML	Refractory relapse	57	M	M	+	−
9,240	NHL	Refractory relapse	48	M	F	+	+
1,046	AML	Refractory relapse	48	M	F	−	−
10,050	AML	Refractory relapse	59	M	F	+	+
9,547	AML	Refractory relapse	58	F	F	+	−
1,056	MDS	RAEB-T	64	F	M	−	−

Abbreviations: Trans, transplant; CMV, cytomegalovirus; NHL, non-Hodgkin's lymphoma; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transition.

PATIENTS AND METHODS

Patient accrual and characteristics. Between November of 1994 and October of 1995, 16 patients with advanced hematologic malignancies received allogeneic PBSC transplants, which were enriched for CD34+ cells, at the Fred Hutchinson Cancer Research Center (FHCRC) or the University of California at Los Angeles (UCLA). Patient and donor characteristics are shown in Table 1. These patients have been observed through June 1, 1996.

Informed consent. Patients and donors were asked to participate in a study evaluating transplants with PBSCs that had been enriched for CD34+ cells as an alternative to BM or unmodified PBSCs. All patients and donors signed informed consent forms approved by the Institutional Review Board of the FHCRC or UCLA. The administration of G-CSF to normal donors was conducted under IND no. 5231 issued by the US Food and Drug Administration.

Donors. Donors were family members who were HLA identical with the patient.[15] All donors received G-CSF (Amgen, Inc, Thousand Oaks, CA) 16 μg/kg/d in 2 subcutaneous injections performed daily for 6 days. PBSCs were collected during a 12 L leukapheresis using a continuous flow blood cell separator (Cobe Laboratories, Lakewood, CO) at a flow rate of 50 to 70 mL/min. Collections were performed on days 4 to 7 with day 4 of G-CSF administration coinciding with recipient day 0.1.

CD34+ cell processing and infusion. PBSCs were processed daily using an avidin-biotin technique (Ceprate Columns; CellPro, Bothell, WA) with the infusion of each CD34+ cell collection without cryopreservation on each of 4 consecutive days.[1] A 4 day separation was used to provide a large number of CD34+ cells in the graft. Because approximately 50% of the CD34+ cells are lost during the separation, 4 selection procedures were chosen to provide approximately the number of CD34+ cells given in 2 days of PBSCs that are unmanipulated. The antibody used for CD34 selection is a mouse monoclonal IgM that has been previously described.[16] Day 0 was the first of 4 consecutive days of CD34+ PBSC infusions.

Treatment regimen. Eleven patients, 10 of whom were less than age 55, received 13.2 Gy and 5 patients older than age 55 received 12.0 Gy of total body irradiation (TBI) given in 1.2 Gy fractions t.i.d. followed by 120 mg/kg of cyclophosphamide given in 2 daily doses of 60 mg/kg. TBI was delivered by dual 60 cobalt sources at a dose rate of 5 to 8 cGy/min for 10 patients (FHCRC) and by a single linear accelerator at a dose rate of 5 to 7 cGy/min for 6 patients (UCLA).

Prophylaxis and treatment of GVHD. Cyclosporine (CSA) was given to all patients for prophylaxis against GVHD. Cyclosporine 5 mg/kg was administered as a continuous intravenous (IV) infusion over 24 hours from day −1 to +3, 3 mg/kg from day +4 through day +14, and 3.75 mg/kg from day +15 through day 35. Patients discharged from the hospital received 5 mg/kg p.o. b.i.d. through day 83 with tapering doses to 2 mg/kg by day 180. After the initial 5 patients were treated with CSA alone, methotrexate (MTX) was added to the prophylactic regimen for the remaining 11 patients; 15 mg/m^2 was given IV on day +4 and 10 mg/m^2 was given on days +6, +9, and +14. Acute GVHD was treated initially with prednisone and failures were treated on protocols in effect at either institution.[17] Chronic GVHD was treated with prednisone and CSA.[18]

Evaluation and definitions. PBSCs were analyzed for CD34+ cells and T-cell subsets by flow cytometry using previously published methods.[19] Engraftment was documented by increasing neutrophil and platelet levels in the blood unsupported by transfusions, by in-situ Y chromosome hybridization of BM or blood samples in sex mismatched donor-recipient pairs and by analysis of variable number tandem repeat (VNTR) polymorphisms in BM and/or blood samples in the case of sex-matched pairs.[20,21] Neutrophil engraftment was defined as the first of 2 consecutive days with an absolute neutrophil count (ANC) >500/μL following the posttransplant nadir. Platelet engraftment was defined as the first of 7 consecutive days with a platelet count >20,000/μL without platelet transfusions. Acute and chronic GVHD were graded as previously described.[22,23]

Patients who died posttransplant in relapse were categorized as dying of relapse irrespective of the proximate cause. Deaths of patients who had not relapsed were categorized as nonrelapse mortality. Infection was listed as the cause of death when bacterial, viral, and fungal infection was the approximate cause of death in the absence of relapse.

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Table 2. Results of CD34+ Selection Procedures

	Cells × 10^6/kg CD34			CD3		CD4		CD8	
UPN	Pre	Post	%CD34+	Pre	Post	Pre	Post	Pre	Post
8,887	21.32	9.02	59	652.17	0.65	520.11	0.50	175.54	0.15
9,141	34.97	17.34	73	557.64	1.08	451.26	0.76	118.76	0.29
6,709	19.36	10.19	56	429.45	2.36	262.93	0.78	187.07	1.66
9,337	31.56	15.30	67	566.42	2.51	341.77	1.03	292.97	1.62
9.426	11.25	6.83	63	556.87	0.76	382.59	0.47	163.95	0.25
733	12.82	6.62	62	576.24	0.86	365.34	0.38	198.74	0.45
1,032	34.24	11.32	75	617.84	0.73	375.62	0.30	257.85	0.35
9,364	14.64	8.90	73	227.73	0.49	201.11	0.37	53.34	0.12
1,036	14.22	4.46	45	544.04	0.41	357.94	0.18	217.86	0.24
1,039	14.45	2.62	34	601.00	0.68	362.15	0.15	202.59	0.55
9,380	19.01	11.81	82	259.71	0.47	194.47	0.29	90.97	0.14
1,046	7.08	3.08	41	378.36	0.67	296.41	0.23	107.05	0.40
9,240	18.26	13.70	75	461.28	0.73	249.40	0.43	212.68	0.26
10,050	16.89	10.48	68	677.77	1.99	361.67	0.70	325.83	1.26
9,547	12.66	8.81	52	572.60	3.65	405.54	0.93	116.17	2.71
1,056	6.74	5.21	59	485.77	0.40	385.52	0.27	192.69	0.17
Median	18.64	8.96	62	557.26	0.73	351.72	0.40	169.74	0.32

RESULTS

Donors. Donors tolerated the injections initially well but moderate bone pain that required acetaminophen with codeine occurred in 10 of 16 donors. Headache with nausea of moderate to severe intensity occurred in 1 donor and required acetaminophen with codeine. This donor also experienced transient elevations of liver function tests with peak SGPT of 257, SGOT 70, SGGT of 90 and alkaline phosphatase of 215. These returned to normal after 2 weeks. The other significant side effect was thrombocytopenia. Platelet counts below 100,000/μL occurred in 8 of 12 donors after the first 3 collections. In 4 donors, by reinfusing platelets contained in the PBSC collection, none of the platelet counts dropped below 100,000.

Results of CD34+ selection. The median number of CD34+ cells collected was 18.64 × 10^6/kg patient body weight and a median of 8.96 × 10^6/kg patient body weight were recovered after processing on avidin-biotin columns (Table 2). The median yield of CD34+ cells was 53% with a 62% median purity. The median number of CD3, CD4, and CD8 cells infused was 0.73, 0.40, and 0.32 × 10^6/kg, respectively, which represented a 2.78 (2.19 to 3.14) log depletion of T cells.

Engraftment. One patient died on day 3 of infection and was unevaluable for recovery of neutrophils (Table 3).[1] The median day to achieve 500 neutrophils/μL was 15 (8 to 26) for the remaining 15 patients. Recovery to >500 neutrophils among those patients not given MTX occurred on day 13, whereas for those receiving MTX recovery occurred at a median of 19 days. Six of 16 patients failed to achieve a platelet count >20,000/μL before death on days 3 to 97 of transplant related causes. The median day to achieve platelets >20,000/mL in the remaining 10 patients was 11 (7 to 31). Platelets recovered earlier in those treated with CSA (day 11) as compared to those treated with CSA and MTX (day 25).

Infections. Four patients had no significant infections during the transplant course. Two patients had clostridium difficile cultured in stool or heliobacter pylori from the stomach. Six patients had transient staphylococcus (n = 4) or enterococcus (n = 2) bacteremia. Four patients died of fatal infections with klebsiella, pseudomonas, aspergillis, and scedasporium prolifican on days 319, 97, 31, and 3, respectively. Two of these deaths were associated with grade 3 acute GVHD or progressive disease.

Chimerism studies. Three patients died early and were unevaluable and data were not obtained before death on day 97 of acute GVHD in 1 additional patient. Informative chimerism data were available from 12 patients. VNTR analysis of BM samples between days 21 to 100 posttransplant showed 99% to 100% donor cells in 6 out of 7 evaluable patients and 40% on day 30 in a patient who died on day 78 of progressive disease. In-situ Y chromosome studies of BM or peripheral blood showed 97% to 99.5% donor cells 30 to 90 days after transplant in 5 out of 5 evaluable patients.

Acute and chronic GVHD. Grade 2-4 acute GVHD occurred in 12 out of 14 (86%) and grades 3-4 in 6 out of 14 (43%) evaluable patients (Table 3). Three of the first 5 patients treated with CSA alone developed grade 3-4 acute GVHD as compared to 3 of 9 evaluable patients given CSA plus MTX. Four of 7 patients age 48 or under had grade 3-4 acute GVHD as compared to 2 out of 7 in patients over the age of 48. Specifics of organ system involvement with GVHD are presented in Table 4. The median days to the development of skin, liver, and gastrointestinal tract involvement were 22, 22, and 25, respectively. Four of 6 evaluable patients developed clinical chronic GVHD and 2 patients developed subclinical chronic GVHD.

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Table 3. Engraftment and GVHD

UPN	Mtx Doses	Day of Engraftment: Days to ANC >0.5 × 10^9/L	Day of Engraftment: Days to PLT >20 × 10^9/L	Transfusions (no.): Platelets	Transfusions (no.): RBC	GVHD: Acute GVHD Grade	GVHD: Chronic GVHD
8,887	—	15	10	24	20	2	Extensive
9,141	—	11	7	36	6	2	Subclinical
6,709	—	8	NR	304	28	3	NE
9,337	—	13	27	58	16	3	NE
9,426	—	14	11	30	16	3	Extensive
733	4	26	31	14	17	2	Limited
1,032	3	12	14	7	12	3	Limited
9,364	2	19	NR	102	12	3	NE
1,036	3	20	16	13	14	1	NE
1,039	0	NR	NR	2	2	NE	NE
9,380	3	19	18	144	35	2	Limited
1,046	2	16	NR	38	26	1	NE
9,240	4	17	14	36	10	2	Extensive
10,050	4	19	NR	194	29	3	NE
9,547	4	14	9	36	11	2	None
1,056	1	19	NR	12	14	NE	NE

Abbreviations: NR, not reached; NE, not evaluable.

The median number of CD3, 4, and 8 positive cells infused in the 8 evaluable patients with grades 1-2 acute GVHD was 0.67 (0.41 to 3.65), 0.38 (0.18 to 0.93), and 0.29 (0.14 to 2.71) × 10^6/kg, respectively, as compared to the 6 patients who had grade 3-4 acute GVHD where the median number of CD3, 4, and 8 positive cells infused were 0.73 (0.49 to 2.51), 0.43 (0.30 to 1.03), and 0.26 (0.12 to 1.66) × 10^6/kg, respectively. Patients given T-cell doses above the median had no more acute GVHD than patients given doses below the median.

Relapse and survival. Seven of 16 patients (44%) died between days 3 and 97 of transplant-related causes and 5 of 16 died of persistent or progressive disease between days 76 to 319. Causes of death are shown in Table 5. Four of 16 (25%) patients are alive and disease-free 312 to 576 days after transplant.

DISCUSSION

Sixteen older patients with advanced hematologic malignancies received allogeneic CD34+ enriched PBSC after high-dose chemo-radiotherapy. All patients were at high-risk for failure of transplantation on the basis of advanced age and disease. PBSCs were procured from HLA-identical sibling donors following G-CSF administration and were then puri-

Table 4. GVHD Organ Involvement

UPN	Skin: Days Post Transplant*	Skin: Maximum Stage/Grade	Liver: Days Post Transplant*	Liver: Maximum Stage/Grade	Gastrointestinal: Days Post Transplant*	Gastrointestinal: Maximum Stage/Grade
8,887	7	2		0	50	1
9,141	22	2		0	25	1
6,709	15	2	50	3	21	3
9,337	13	2	9	2	23	1
9,426	14	3	14	3	12	1
733	29	1		0	30	1
1,032	24	1	27	2	27	1
9,364	33	2	40	2		1
1,036		0		0	26	1
1,039		NE		NE		NE
9,380	8	3	12	2	6	2
1,046	25	2		0		0
9,240	12	2		0	13	1
10,050	10	3	43	4	43	2
9,547	32	1	17	1	31	1
1,056		NE		NE		NE

Abbreviation: NE, not evaluable.

* Day GVHD first developed.

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Table 5. Outcome of CD34+ Cell Transplants

UPN	Date of Transplant	Relapse	Survival	Cause of Death
8,887	11/01/94		580*	
9,141	11/03/94		578*	
6,709	01/24/95		55	Pulmonary hemorrhage
9,337	01/24/95		27	Bowel perforation
9,426	01/31/95	135	242	Progressive disease
733	04/04/95		427*	
1,032	05/02/95	246	319	Progressive disease
9,364	05/03/95		97	Renal failure
1,036	05/23/95		76	Persistent disease
1,039	06/06/95		3	Septic shock
9,380	07/18/95	107	161	Progressive disease
1,046	07/25/95		31	Fungal infection
9,240	07/25/95		316*	
10,050	08/15/95		63	VOD/Renal failure
9,547	09/01/95	81	122	Progressive disease
1,056	10/10/95		19	VOD, Multi-organ system failure

* Surviving.

fied by positive selection for the CD34 antigen. Purification of early hematopoietic progenitors, with a resultant 2 to 3 log depletion of T cells, resulted in rapid and complete reconstitution of hematopoiesis without graft failure. The rate of hematopoietic recovery appeared to be somewhat faster than seen with marrow when patients receiving similar forms of GVHD prophylaxis are compared.

Despite the reduced number of residual T cells infused (0.43 to 3.65 $\times$ 10^6/kg), the incidence of grade 2-4 acute GVHD was high (12 out of 14 evaluable patients). In a series of 41 unselected PBSC allografts, Körbling et al[12] infused a median of 393, 257, and 154 $\times$ 10^6 CD3, CD4, and CD8 cells, respectively; approximately 3 log higher nembers of T cells than were given in the present study. Despite the 1,000-fold greater numbers of T cells acute GVHD was observed in only 48% of patients. These numbers compared with approximately 1 log fewer T cells given with unmanipulated marrow and no difference in the incidence of acute GVHD.

Grade 3-4 acute GVHD occurred in 3 out of 5 patients given CSA alone and GVHD prophylaxis was changed in subsequent patients to the addition of MTX. In the next cohort, 3 out of 9 patients given CSA plus MTX developed grades 3-4 acute GVHD suggesting that the added MTX may have decreased the incidence of severe acute GVHD. It should be noted that because PBSC were given on 4 consecutive days the first dose of MTX was not given until day 4, 3 days after the first PBSCs were infused. This may have interfered with the effectiveness of MTX. The median number of T cells infused into recipients who developed grade 1-2 acute GVHD or grade 3-4 acute GVHD did not differ. In addition, 6 out of 8 evaluable patients developed clinical chronic GVHD.

Although the side effects of G-CSF administration to normal donors was tolerable, there was a high incidence of moderate to severe bone pain requiring codeine for relief. In addition it was noted that significant thrombocytopenia occurred primarily from platelet losses in the collection product, because the re-infusion of platelets salvaged from the stem cell product prevented a further drop in platelet levels.

These data are consistent with those reported by Link et al[14] where 3 out of 5 patients transplanted with CD34 selected allogeneic PBSC and CSA alone for prophylaxis of GVHD developed grade 3-4 acute GVHD, with 2 deaths from acute GVHD; all 3 surviving patients developed chronic GVHD. The median grade of acute GVHD for 5 similar patients given CSA plus MTX was 1 and 2 out of 5 developed chronic GVHD. In another study from our institution, using an immunomagnetic bead technique, all 6 patients transplanted with CD34 selected PBSC followed by CSA alone for GVHD prophylaxis developed grade 2 or 3 acute GVHD.[24] These data contrast to those of Finke et al[25] where allogeneic transplantation with CD34 selected PBSCs, which were cryopreserved, resulted in grade 2 or 3 acute GVHD, limited to the skin, in only 2 out of 8 patients given CSA alone for GVHD prophylaxis. It is possible that cryopreservation modified the alloreactivity of the graft in this study.[25]

Older patients were deliberately selected for this study because of the concern about a higher incidence and severity of GVHD in this patient population receiving unmodified BM transplants.[26] If older patients get more acute GVHD than younger patients, this could partially explain the high incidence of acute GVHD seen in this study despite the removal of 2 to 3 logs of T cells. Because only limited numbers of patients over age 50 have received allogeneic transplants, the exact increase in GVHD associated with advanced age is not known with certainty. In the current study, grade 3-4 acute GVHD was not more frequent in patients older than the median age of 48, suggesting that age was unlikely to be the entire explanation for the high incidence of GVHD. However, it is worth noting that the initial patient transplanted on this study (UPN 8887) is alive and well 580 days after transplant and may well be the oldest patient to receive an allogeneic transplant.

In a concurrent study of unmodified allogeneic PBSC in younger patients (median age of 38) the infusion of 3 logs more T cells than with BM transplantation was associated with a 37% and 14% incidence of grades 2-4 and 3-4 acute GVHD, respectively.[27] This incidence of acute GVHD is no higher than would be anticipated following marrow transplantation showing that the infusion of large numbers of lymphocytes contained in G-CSF mobilized PBSC did not increase the incidence or severity of acute GVHD. Whether unmodified allogeneic PBSCs would have caused as high or a higher rate of acute GVHD than CD34+ selected cells in patients with an age distribution of the present study is unknown.

The median dose of CD3 cells infused in this study was 0.73 $\times$ 10^6/kg, which is well above the 10^5/kg dose of T cells identified as the threshold for development of grade 1-2 acute GVHD following marrow transplantation when no posttransplant immunosuppression was used.[4,5] In previous studies T-cell doses up to 4.4 $\times$ 10^5/kg were not associated with any episodes of grade 3-4 acute GVHD.[28] In another

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study, patients given a fixed dose of 1×10^5/kg of T cells, followed by posttransplant immunosuppression with CSA, had no episodes of acute GVHD.[29] Wagner et al[30] suggest that a T-cell dose below 5×10^5/kg is not associated with severe acute GVHD when CSA is used for prophylaxis. In the current study, the lowest T-cell dose administered was 4×10^5/kg and only 4 patients received less than 5×10^5 CD3 cells/kg and all developed grade 2-4 acute GVHD. Because the majority of patients received CD3 cell doses above 5×10^5/kg it may be necessary to further reduce the numbers of T cells in the CD34 enriched product by a second depletion step.

An alternative explanation for the relatively high incidence of acute and chronic GVHD after CD34 selection could be the removal of T cells or other cells from G-CSF mobilized PBSCs that reduce alloreactivity of the graft. Support for this hypothesis comes from studies in mice where G-CSF induces type 2 lymphocytes to produce less γ-interferon and interleukin-2.[31] In this mouse model, transplants from G-CSF treated animals had less GVHD than animals receiving grafts from control animals. Studies of GM-CSF mobilized PBSCs collected for autologous transplantation have shown irradiation resistant suppressor cell activity in the PBSC product.[32] In this same study there was also an increase in $CD4^-$, $CD8^-$, TCR α/β^+ cells, a phenotypic homolog of natural suppressor cells, in the peripheral blood following transplantation of autologous PBSCs. These findings may help explain the lack of expected increase in GVHD with unmodified allogeneic PBSC transplants and could help explain why $CD34^+$ selection failed to decrease GVHD.

In conclusion, CD34 selected allogeneic PBSCs, in doses that are 3 times the usual BM $CD34^+$ cell dose, in older, high-risk patients with advanced hematologic malignancies provided rapid and sustained allogeneic reconstitution without graft-failure. However, $CD34^+$ selection did not prevent acute and chronic GVHD from developing in most patients.

REFERENCES

1. Bensinger WI, Weaver CH, Appelbaum FR, Rowley S, Demirer T, Sanders J, Storb R, Buckner CD: Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 85:1655, 1995

2. Körbling M, Przepiorka D, Huh YO, Engel H, van Besien K, Giralt S, Andersson B, Kleine HD, Seong D, Deisseroth AB, Andreeff M, Champlin R: Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: Potential advantage of blood over marrow allografts. Blood 85:1659, 1995

3. Schmitz N, Dreger P, Suttorp M, Rohwedder EB, Haferlach T, Löffler H, Hunter A, Russell NH: Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (G-CSF). Blood 85:1666, 1995

4. Kernan NA: T-cell depletion for prevention of graft-versus-host disease, in Forman SJ, Blume KG, Thomas ED (eds): Bone Marrow Transplantation. Boston, MA, Blackwell Scientific, 1994, p 124

5. O'Reilly RJ, Kernan N, Cunningham I, Brochstein J, Collins N, Laver J, Castro-Malaspina H, Flomenberg N, Gulati S, Emanuel D, Bordignon C, Keever C, Shank MB: Soybean lectin agglutination and E-rosette depletion for removal of T-cells from HLA-identical and non-identical marrow grafts administered for the treatment of leukemia, in Martelli MF, Grignani F, Reisner Y (eds): T-Cell Depletion in Allogeneic Bone-Marrow Transplantation. Rome, Italy, Ares-Serono Symposia, 1988, p 123

6. Bolger GB, Sullivan KM, Storb R, Witherspoon RP, Weiden PL, Stewart P, Sanders J, Meyers JD, Martin PJ, Doney KC, Deeg HJ, Clift RA, Buckner CD, Appelbaum FR, Thomas ED: Second marrow infusion for poor graft function after allogeneic marrow transplantation. Bone Marrow Transplant 1:21, 1986

7. Anasetti C, Martin PJ, Storb R, Hansen JA: Engraftment of allogeneic hematopoietic stem cells in patients conditioned only with anti-CD3 monoclonal antibody BC3 plus methylprednisolone. Blood 84:249a, 1994 (abstr, suppl 1)

8. Aversa F, Tabilio A, Terenzi A, Velardi A, Falzetti F, Giannoni C, Iacucci R, Zei T, Martelli MP, Gambelunghe C, Rossetti M, Caputo P, Latini P, Aristei C, Raymondi C, Reisner Y, Martelli MF: Successful engraftment of T-cell-depleted halpoidentical "Three-Loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 11:3948, 1994

9. DiPersio J, Martin B, Abboud C, Ryan D, Berenson R: Allogeneic BMT using bone marrow and CD34-selected mobilized PBSC; comparison to BM alone and mobilized PBSC alone. Blood 84:91a, 1994 (abstr, suppl 1)

10. Dreger P, Viehmann K, Steinmann J, Eckstein V, Müller-Ruchholtz W, Löffler H, Schmitz N: G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: Comparison of T cell depletion strategies using different CD34+ selection systems or CAMPATH-1. Exp Hematol 23:147, 1995

11. Arseniev L, Kadar JG, Berenson RJ, Battmer K, Sudmeier I, Casper J, Diedrich H, Stangel W, Link H: Allogeneic peripheral blood progenitor cell mobilization, collection and immunoselection. Bone Marrow Transplant 15:S5, 1995 (abstr, suppl 2)

12. Körbling M, Huh YO, Durett A, Mirza N, Miller P, Engel H, Anderlini K, van Besien K, Andreeff M, Przepiorka D, Deisseroth AB, Champlin RE. Allogeneic blood stem cell transplantation: Peripheralization and yield of donor-derived primitive hematopoietic progenitor cells ($CD34^+$ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host-disease. Blood 86:2842, 1995

13. Yeager AM, Holland HK, Mogul MJ, Forte K, Lauer M, Boyer MW, Turner C, Vega RA, Beatty PG, Jacobs CA, Benyunes MC, Wingard JR: Transplantation of positively selected $CD34^+$ cell from haploidentical parental donors for relapsed acute leukemia in children. Blood 86:1149, 1995 (abstr, suppl 1)

14. Link H, Arseniev L, Bahre O, Kadar JG, Battmer K, Casper J, Diedrich H: Transplantation of allogenic hematopoiesis by selected $CD34^+$ blood cells. Blood 86:1150, 1995

15. Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, Buckner CD, Clift R, Doney K, Martin PJ, Mickelson E, Nisperos B, O'Quigley J, Ramberg R, Sanders JE, Stewart P, Storb R, Sullivan KM, Witherspoon RP, Thomas ED, Hansen JA: Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. N Engl J Med 320:197, 1989

16. Andrews RG, Singer JW, Bernstein ID: Monoclonal antibody 12.8 recognizes a 115-kd molecule present on both unipotent and multipotent hematopoietic colony-forming cells and their precursers. Blood 67:842, 1986

17. Martin PJ, Schoch G, Fisher L, Byers V, Anasetti C, Appelbaum FR, Beatty PG, Doney K, McDonald GB, Sanders JE, Sullivan KM, Storb R, Thomas ED, Witherspoon RP, Lomen P, Hannigan J, Hansen JA: A retrospective analysis of therapy for acute graft-versus-host disease: Initial treatment. Blood 76:1464, 1990

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18. Sullivan KM, Gooley T, Nims J, Flowers M, Lin D, Anasetti C, Sanders J, Witherspoon R, Appelbaum FR, Storb R: Comparison of cyclosporine (CSP), prednisone (PRED) or alternating-day CSP/PRED in patients with standard and high-risk chronic graft-versus-host disease (GVHD). Blood 82:845, 1993 (abstr, suppl 1)

19. Bensinger WI, Longin K, Appelbaum F, Rowley S, Weaver C, Lilleby K, Gooley T, Lynch M, Higano T, Klarnet J, Chauncey T, Storb R, Buckner CD: Peripheral blood stem cells (PBSCs) collected after recombinant granulocyte colony stimulating factor (rhG-CSF): An analysis of factors correlating with the tempo of engraftment after transplantation. Br J Haematol 87:825, 1994

20. Kirk JA, VanDavantar DR, Biberman J, Bryant EM: Y chromosome loss in chronic myeloid leukemia detected in both normal and malignant cells by interphase fluorescence in situ hybridization. Genes Chromosomes Cancer 11:141, 1994

21. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC: Analysis of the VNTR locus DIS80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 48:137, 1991

22. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED: Consensus conference on acute GVHD grading. Bone Marrow Transplant 15:825, 1995

23. Siadak M, Sullivan KM: The management of chronic graft-versus-host disease. Blood Rev 8:154, 1994

24. Bensinger WI, Rowley S, Appelbaum FR, Mills B, Oldham F, Chauncey T, Buckner CD: CD34 selected allogeneic peripheral blood stem cell (PBPC) transplantation in older patients with advanced hematologic malignancies. Blood 86:97a, 1995 (abstr, suppl 1)

25. Finke J, Brugger W, Einsele H, Bertz H, Behringer D, Kanz L, Mertelsmann R: Allogeneic transplantation of peripheral blood derived $CD34^+$ selected progenitor cells from matched related donors: Results of a clinical phase I/II study. Blood 86:290a, 1995 (abstr, suppl 1)

26. Nash RA, Pepe MS, Storb R, Anasetti C, Appelbaum FR, Deeg HJ, Doney K, Martin PJ, Sullivan KM, Witherspoon RP: Risk factors for acute graft-versus-host disease associated with cyclosporine and methotrexate prophylaxis. Blood 81:1973, 1993

27. Bensinger WI, Clift R, Martin P, Appelbaum FR, Demirer T, Gooley T, Lilleby K, Rowley S, Sanders J, Storb R, Bucker CD: Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: A retrospective comparison with marrow transplantation. Blood (in press)

28. Kernan NA, Collins NM, Juliano L, Cartagenia T, Dupont B, O'Reilly RJ: Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-versus-host disease. Blood 68:770, 1986

29. Verdonck LF, Dekker AW, de Gast GC, van Kempen ML, Lokhorst HM, Nieuwenhuis HK: Allogeneic bone marrow transplantation with a fixed low number of T cells in the marrow graft. Blood 83:3090, 1994

30. Wagner JE, Santos GW, Noga SJ, Rowley SD, Davis J, Vogelsang GB, Farmer ER, Zehnbauer BA, Saral R, Donnenberg AD: Bone marrow graft engineering by counterflow centrifugal elutriation: Results of a phase I-II clinical trial. Blood 75:1370, 1990

31. Pan L, Delmonte J Jr, Jalonen CK, Ferrara JLM: Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft versus host disease. Blood 86:4422, 1995

32. Talmadge JE, Reed EC, Kessinger A, Kuszynski CA, Perry GA, Gordy CL, Mills KC, Thomas ML, Pirruccello SJ, Letheby BA, Arneson MA, Jackson JD: Immunologic attributes of cytokine mobilized peripheral blood stem cells and recovery following transplantation. Bone Marrow Transplant 17:101, 1996

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