Table 1 Patient, donor and graft characteristics

	Manipulation		
	No. (n = 18)	*CD34+ cell selection (n = 32)*	
		CliniMACS (n = 17)	*Isolex (n = 15)*
Median age (range) (years)			
Patients	32 (20–55)	35 (18–69)	29 (17–48)
Donors	42 (16–60)	42 (18–59)	44 (21–67)
Gender match, donor/patient			
Male/male	4[a]	8	8
Female/male	5	5	3
Female/female	8	2	2
Male/female	1	2	2
Relationship of donor to patient			
Father/mother	1/7	2/2	3/2
Sibling	5	9	9
Son/daughter	0/5	3/1	0/0
Aunt	0	0	1
Genotypic disparities			
GVH direction			
HLA-A/-B and -DRB1	1/9	1/7	1/3
HLA-A, -B and -DRB1	8	9	11
HVG direction			
HLA-A/-B/-DRB1	0/0/0	0/1/2	1/0/0
HLA-A and -B	0	1	1
HLA-A/-B and -DRB1	1/10	1/5	1/3
HLA-A, -B and -DRB1	7	7	9
Disease at transplant			
AML	2	9	6
ALL	1	2	3
MDS	4	0	2
CML	4	3	1
NHL	6	2	3
MM	1	1	0
Prior autologous PBSCT/allogeneic HSCT	3/4	2/3	5/0
Median time interval[b] (range) (months)	10.5 (0.2–24.2)	4.0 (0.4–96.8)	1.0 (0.1–38.1)
ECOG PS 0/1/2/3	3/8/5/2	5/8/3/1	6/7/1/1
Median graft size (range)[c]			
Nucleated cell dose ($\times 10^7$/kg)	78.2 (11.3–324.0)	0.71 (0.36–1.4)	0.38 (0.19–0.56)
CD34+ cell dose ($\times 10^6$/kg)	4.2 (1.5–9.5)	6.8 (2.9–13.5)	4.4 (0.67–9.8)
CD3+ cell dose (/kg)	2.7 (1.3-5.4) $\times 10^8$	2.8 (0.30–5.0) $\times 10^4$	4.0 (1.7–24.7) $\times 10^4$
Median follow-up (range) (months)	2.5 (0.10–15.4)	3.8 (0.20–16.8)	2.7 (0.30–35.5)

[a]Number of patients unless indicated otherwise.
[b]Time from diagnosis to transplant.
[c]The dose of CD34+ cells was significantly higher in patients receiving CD34+ blood cells purified by a CliniMACS device than in those receiving unmanipulated PBSC ($P = 0.0027$) or CD34+ blood cells purified by an Isolex system ($P = 0.014$). The dose of CD3+ cells was significantly higher in patients receiving unmanipulated PBSC than in those receiving CD34+ blood cells purified by a CliniMACS device ($P = 0.0004$) or an Isolex system ($P = 0.0009$).GVH, graft-versus-host; HVG, host-versus-graft; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; ECOG PS, Eastern Cooperative Oncology Group performance score.

among 25 patients with sustained engraftment for ⩾100 days after transplant.

Regimen-related toxicity (RRT) of organ systems before day 100 post transplant was graded according to the criteria proposed by Bearman *et al.*[19] A clinical diagnosis of veno-occlusive disease (VOD) was made based on the presence of jaundice (bilirubin ⩾2 mg/dl), hepatomegaly and/or right upper quadrant pain, and ⩾5% weight gain from admission, with or without ascites.[20] The diagnosis of thrombotic microangiopathy (TMA) was made if the patient had thrombocytopenia (defined as a platelet count $<100 \times 10^9$/l), microangiopathic hemolytic anemia as indicated by red blood cell fragmentation present in a peripheral blood smear and elevated lactate dehydrogenase (LDH), without an identifiable cause for the thrombocytopenia or microangiopathic hemolytic anemia (eg, sepsis, disseminated intravascular coagulation, carcinoma, eclampsia).[21]

Relapse was defined either by morphologic evidence of the disease in the peripheral blood, marrow or extramedullary sites, or by recurrence and sustained presence of pretransplant chromosomal abnormalities in cytogenetic analysis of the marrow cells. Patients with CML, in whom the sole evidence of the disease was positivity for the bcr/abl RNA transcript by polymerase chain reaction, were not classified as having relapse. Both relapse and progression were defined as disease progression with transplant-related deaths being censored. Treatment-related mortality (TRM)

Table 2 Treatment characteristics

	Manipulation		
	No. (n = 18)	CD34+ cell selection (n = 32)	
		CliniMACS (n = 17)	Isolex (n = 15)
Conventional conditioning regimen	7 (39%)[a]	9 (53%)	15 (100%)
TBI + CY + others[b]/TBI + melphalan	3/0	8/1	14/0
BU + CY + others[c]	4	0	1
ATG-containing	1 (6%)	0	7 (47%)
GVHD prophylaxis			
CYA + MTX/CYA + prednisolone/CYA	1/0/0	5/0/1	7/2/2
FK506 + MTX/FK506	6/0	3/0	0/4
Reduced-intensity conditioning regimen	11 (61%)	8 (47%)	0
TBI + CY/TBI + Flu + BU/TBI + Flu + ATG + others[d]/TBI + BU + ATG	1/0/0/0	0/2/4/1	0/0/0/0
Flu + others[e]	10	1	0
ATG-containing	6 (33%)	5 (29%)	0
GVHD prophylaxis			
CYA + MTX/CYA + prednisolone/CYA + MMF/CYA	1/1/0/1	0/0/3/2	0/0/0/0
FK506 + MTX/FK506 + prednisolone + MMF/FK506 + prednisolone/FK506	6/1/1/0	0/0/0/1	0/0/0/0
Prednisolone/none	0/0	1/1	0/0
G-CSF after transplant	12 (67%)	16 (94%)	14 (93%)

[a]Number of patients (%) unless indicated otherwise.
[b]Others = ATG, BU, Ara-C, thiotepa or VP-16.
[c]Others = ATG, Ara-C, Flu or melphalan.
[d]Others = BU, CY or thiotepa.
[e]Others = BU, CY, Ara-C, idarubicin or melphalan.
ATG, antithymocyte globulin; Flu, fludarabine; MMF, mycophenolate mofetil.

was defined as death from any cause other than relapse or disease progression. Progression-free survival (PFS) was defined as the time interval from transplant to the first event including relapse, disease progression and death. Overall survival (OS) was defined as the time interval from transplantation to death.

Data collection

Questionnaires were returned by 216 of 432 (50%) transplant centers in Japan.

A total of 91 adult patients underwent allogeneic HSCT from a genotyped related donor mismatched at two or three loci in the GVH direction for the treatment of hematologic malignancies in a total of 32 centers (listed in the Appendix).[22] These data were collected from medical records. Histocompatibility was determined by serology for HLA-A, -B and -DR antigens and/or by DNA typing for HLA-A, -B and -DRB1. All of these HLA data were reviewed, and inquiries concerning patient and donor HLA typings were verified by the central committee. Of these 91 patients, 24 were excluded because they received BM, five because they were thought not to be at high risk for relapse or refractory to intensive chemotherapy, and six because their donors were not anticipated to be serological two- or three-loci-MMRD. As in allogeneic HSCT from related as well as unrelated donors, both HLA class I and II gene disparities contribute to increased incidence of graft failure or acute GVHD prophylaxis,[23,24] identification of the fact that both HLA class I and II genes were disparate would be helpful. Thus, six patients were excluded because they received allogeneic PBSC from a two- or three-loci-MMRD with only HLA class I or II gene disparity in the GVH direction. Finally, we confirmed that 50 evaluable patients underwent allogeneic PBSCT from a two- or three-loci-MMRD with HLA class I (HLA-A and/or -B) and II (HLA-DRB1) gene disparities in the GVH direction for high-risk hematologic malignancies in 20 centers.

Statistical analysis

Comparisons of variables were performed using the two-tailed Fisher exact test or the χ^2 test. Continuous variables were compared by the Mann–Whitney U-test or the Kruskal–Wallis test. Competing risks for grades II–IV acute GVHD included death without grades II–IV acute GVHD, relapse, and graft failure and rejection. Relapse was a competing risk for TRM at day 100 post transplant, and TRM was a competing risk for relapse. The association of variables with the outcome was evaluated in multivariable analyses, with the use of stepwise Cox regression to adjust for differences in potentially confounding variables.[25–27] The variables considered were age and gender of patients or donors, number of transplantations, performance status (PS, which was evaluated before the start of the conditioning regimen and graded according to the Eastern Cooperative Oncology Group (ECOG) performance score), CD34+ cell doses, degree of HLA genotypic disparity in the GVH direction, conditioning regimens including reduced regimens, TBI and ATG used, GVHD

prophylaxis and G-CSF used after transplant. Each of these factors was checked for the assumption of proportional hazards by using a time-dependent covariate. The interactions with outcomes, such as the incidence of neutrophil and platelet engraftment, complications including infection before day 100 post transplant, grades II–IV acute GVHD and TRM at day 100 post transplant, were assessed in a model that included these outcomes and the variables under consideration. Only factors significantly associated with the outcomes ($P<0.05$) were retained in the final models. End points were calculated on the day of the last patient contact. The outcomes PFS and OS following transplant were estimated by the Kaplan–Meier method and significance assessed by the log-rank test ($P<0.05$).

Results

Graft characteristics

The dose of CD34+ cells given was significantly higher in patients who received CD34+ blood cells purified by a CliniMACS device (CliniMACS-purified CD34+ cells, $n=17$) than in those who received unmanipulated PBSC ($n=18$; $P=0.0027$) or CD34+ blood cells purified by an Isolex system (Isolex-purified CD34+ cells, $n=15$; $P=0.014$; Table 1). The target dose of CD34+ cells (3.0×10^6 CD34 cells/kg) was achieved in 36 donors (72%) by performing two or more leukaphereses. In all, 14 patients (28%) received $<3.0\times10^6$ CD34 cells/kg in unmanipulated PBSC ($n=7$), or CliniMACS-purified CD34+ cells ($n=1$) or Isolex-purified CD34+ cells ($n=6$). The dose of CD3+ cells was also significantly higher in those receiving unmanipulated PBSC than in those receiving CliniMACS- or Isolex-purified CD34+ cells ($P=0.0004$ or 0.0009, respectively). As the Isolex systems have changed over the years, variable CD34+ cell doses from the Isolex system may be related to the use of earlier or later version of this equipment.

Engraftment

Among 39 patients (78%) who survived for ⩾28 days after transplant, 37 (95%) and 25 (64%) achieved neutrophil and platelet engraftment, respectively. There was no significant difference in the number of days required for neutrophil engraftment between patients receiving unmanipulated PBSC (median 14 days, range 10–27 days), CliniMACS- and Isolex-purified CD34+ cells (14, 9–20 and 12, 9–20, respectively). There was also no significant difference in the number of days required for platelet engraftment between patients receiving unmanipulated PBSC (18.5, 0–46 days), and CliniMACS- and Isolex-purified CD34+ cells (14, 9–23 and 16, 12–37, respectively).

A total of 11 patients (22%) died before 28 days (median 12 days range 3–27 days) post transplant without evidence of engraftment. Of these patients, two receiving $<3.0\times10^6$ CD34 cells/kg of unmanipulated PBSC or Isolex-purified CD34+ cells died of RRT.

Graft failure and rejection

Graft failure occurred in two patients (5%). One of them had received CliniMACS-purified CD34+ cells from a three-loci-MMRD, mismatched for HLA-A, -B and -DR antigens in the HVG direction, and was given a reduced-intensity conditioning regimen containing ATG and GVHD prophylaxis with prednisolone. This patient developed grade IV pulmonary toxicity and died of pulmonary hemorrhage. The other patient with graft failure received Isolex-purified CD34+ cells from a two-loci-MMRD, mismatched for HLA-B and -DR antigens in the HVG direction, and was given a conventional conditioning regimen containing ATG and GVHD prophylaxis with CYA plus prednisolone. This patient developed grade III stomatitis and gastrointenstinal toxicity and died of TMA after secondary allogeneic BMT. Additionally, they received purified CD34+ cells mismatched for HLA-C antigen in the HVG direction.

Three patients (8%) developed graft rejection. All of them had received CliniMACS-purified CD34+ cells and a conventional conditioning regimen including 12 Gy TBI without ATG. One patient received purified CD34+ cells of $<3.0\times10^6$ CD34 cells/kg. Their donors were all mismatched for HLA-B and -DR antigens in the HVG direction, of whom two were also mismatched for HLA-C + -A antigens in the HVG direction. They received GVHD prophylaxis with CYA plus methotrexate ($n=2$) or CYA alone ($n=1$).

GVHD

Grades II–IV acute GVHD developed in nine (64%) of 14 evaluable patients receiving unmanipulated PBSC, in three (20%) of 15 receiving CliniMACS-purified CD34+ cells and five (36%) of 14 receiving Isolex-purified CD34+ cells. By stepwise Cox regression analysis, the incidence of grades II–IV acute GVHD in patients receiving purified CD34+ cells (28%) was significantly lower than in those receiving unmanipulated PBSC (64%) (hazard ratio (HR) 0.32; 95% confidence interval (CI) 0.12–0.84; $P=0.022$). While no patients receiving CliniMACS- ($n=4$) or Isolex- ($n=7$) purified CD34+ blood cells with conditioning regimens containing ATG developed grade III or IV acute GVHD, four of 18 patients (22%) receiving CliniMACS- ($n=11$) or Isolex- ($n=7$) purified CD34+ cells without ATG developed grade III or IV acute GVHD. Chronic GVHD developed in three of the 25 evaluable patients (12%; Table 3).

Regimen-related toxicity

Six patients (33%) of those receiving unmanipulated PBSC and 13 (41%) of those receiving purified CD34+ cells developed RRT including grades II–IV organ toxicity before day 100 post transplant according to Bearman's criteria. Additionally, two patients (11%) and five (28%) of those receiving unmanipulated PBSC, and one (3%) and three (9%) of those receiving purified CD34+ cells developed VOD or TMA, respectively (Table 3). Seven of

Table 3 Engraftment, GVHD and regimen-related toxicity

	Manipulation		
	No. (n = 18)	CD34+ cell selection (n = 32)	
		CliniMACS (n = 17)	Isolex (n = 15)
Median time of engraftment (range) (days)			
Neutrophil	14 (10–27)	14 (9–20)	12 (9–20)
Platelet	18.5 (0–46)	14 (9–23)	16 (12–37)
Graft failure/rejection	0[a]/0	1/3	1/0
Acute GVHD[b]			
0/I	3/2	9/3	5/4
II/III/IV	1/6/2	2/0/1	2/2/1
Median onset (range) (days) of ≥II acute GVHD	14 (6–77)	26.5 (3–50)	12.5 (5–32)
Chronic GVHD[c] *(onset, days)*			
None/limited/extensive	7/1 (105)/0	9/0/1 (112)	6/1 (101)/0
RRT[d] II/III/IV	2/2/2	1/1/2	2/6/1
VOD/TMA	2/5	1/1	0/2

[a]Number of patients unless indicated otherwise.
[b]A total of 43 patients who developed acute GVHD within 28 days or who survived ≥28 days after transplant were evaluated for acute GVHD.
[c]A total of 25 patients who engrafted and survived ≥100 days after transplant were evaluated for chronic GVHD.
[d]Maximum early RRT was graded according to the criteria documented by Bearman *et al*.RRT, regimen-related toxicity; VOD, veno-occlusive disease; TMA, thrombotic microangiopathy; ≥II acute GVHD, grades II–IV acute GVHD.

11 (64%) patients receiving unmanipulated PBSC and eight of 14 (57%) receiving purified CD34+ cells died of RRT.

Infectious complications

By day 100 post transplant, the most serious treatment-related problem was some form of severe infectious complication, which was seen in 11 patients (61%) receiving unmanipulated PBSC, as follows: bacterial sepsis ($n=8$, 44%), bacterial pneumonia ($n=3$, 17%), fungal infection ($n=2$, 11%) including Candida ($n=1$) and others ($n=1$), and adenoviral disease ($n=2$, 11%). In 20 patients (63%) receiving purified CD34+ cells, these figures were as follows: bacterial sepsis ($n=12$, 39%), bacterial pneumonia ($n=2$, 6%), fungal infection ($n=10$, 31%) including Aspergillus ($n=2$), Candida ($n=1$) and others ($n=7$), and viral disease ($n=10$, 31%) including CMV ($n=5$) and adenoviral ($n=3$) disease, and VZV reactivation ($n=2$).

Five of these 11 patients (45%) receiving unmanipulated PBSC and eight of these 20 (40%) receiving purified CD34+ cells died of infectious complications before day 100 post transplant. Four of these five patients (80%) receiving unmanipulated PBSC and two of these eight (29%) receiving purified CD34+ cells suffered from infectious complications before achieving neutrophil engraftment. Two patients, who developed grade IV acute GVHD and died of acute GVHD, suffered the complication of Candida infection or CMV disease, respectively, before day 100 post transplant (Table 4).

Eight patients (16%) had carried over active infection to transplant. By day 100 post transplant, three patients who had bacterial infections (methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* or *Stentrophomonas maltophilia*) (infectious sites unknown) before the start of the conditioning regimen suffered sepsis, of whom one died of *P. aeruginosa* sepsis and one of adenoviral pneumonia after recovery from MRSA sepsis. One patient who had pneumonia of unknown origin died of bacterial sepsis, also of unknown origin. Two patients who had Candida liver abscesses or infections of unknown origin and site had *Staphylococcus hemolyticus* or bacterial sepsis of unknown origin, respectively (but recovered). One patient who had active chronic hepatitis C infection died of VOD. One patient who had *Staphylococcus epidermidis* tonsillitis had no infectious complications after transplantation.

Cause of death, relapse, disease progression and survival

In all 11 patients are surviving at a median of 11.7 months (range 4.4–35.5 months) and 39 patients died at a median time of 67 days (range 3–254 days) after transplant. The primary causes of death up to 1 year post transplant in 39 patients (78%) are listed in Table 5. The most common cause of death was treatment-related problem, which was seen in 28 patients (56%). Of the 39 patients who survived ≥28 day after transplant with confirmed evidence of engraftment, two of 13 (15%) receiving unmanipulated PBSC and two of 26 (8%) receiving purified CD34+ cells relapsed before day 100 post transplant.

Six patients were treated for relapse or disease progression with donor lymphocyte infusion (DLI), the median dose of CD3+ cells being 9.6×10^6/kg (range 1.5×10^5–6.6×10^7/kg), and one underwent a third allogeneic PBSCT. Although grades II acute GVHD developed in two patients who received DLI, and grade III in one, there was no noticeable effect of DLI on disease progression.

Within 168 days after transplant, two of seven patients (29%) undergoing allogeneic PBSCT after prior allogeneic HSCT relapsed and received DLI. One of these two died of

Table 4 Infectious complications before day 100 post-transplant

	Manipulation							
	No. (n = 18)				*CD34+ cell selection (n = 32)*			
	Total	*Neut*	*aGVHD*	*Both*	*Total*	*Neut*	*aGVHD*	*Both*
Bacterial								
Sepsis	8 (2)[a]	6 (2)	1	0	12 (3)	6 (1)	1	0
Pneumonia	3 (1)	3 (1)	0	0	2 (2)	0	0	0
Fungal								
Aspergillus	0	0	0	0	2	1	0	0
Candida	1 (1)	0	1 (1)	0	1	0	0	0
Others[b]	1 (1)	1 (1)	0	0	7 (1)	3 (1)	2	1
Viral								
CMV disease	0	0	0	0	5 (2)	0	1 (1)	0
Adenoviral disease	2	0	1	0	3	0	0	0
VZV reactivation	0	0	0	0	2	1	0	0
CMV antigenemia	6	1	0	3	15	7	0	3

[a]Number of patients (number of deaths) is shown.
[b]Others = *Rhodotorula rubra, Pneumocystis carinii* or origin unknown.
Neut, number of patients with incidence of infectious complications before achieving neutrophil engraftment; aGVHD, number of patients with incidence of infectious complications after treating grades II–IV acute GVHD with prednisolone; both, number of patients with incidence of infectious complications before achieving neutrophil engraftment (Neut) and after treating grades II–IV acute GVHD with prednisolone (aGVHD); CMV antigenemia, positive for CMV antigen without diseases caused by CMV infection.

Table 5 Causes of death before 1 year post transplant

	Manipulation	
	No. (n = 18)	*CD34+ cell selection (n = 32)*
Relapse/progressive disease	2 (11%)	9 (28%)
Treatment-related problem	11 (61%)	17 (53%)
Infectious complication	5 (28%)	10 (31%)
Organ toxicity[a]	5 (28%)	5 (16%)
Acute GVHD + infectious complication	1 (5%)	2 (6%)

[a]Organ toxicity = pulmonary hemorrhage (*n* = 2), VOD (*n* = 2), TMA (n = 2), interstitial pneumonia (*n* = 2), intracerebral hemorrhage (*n* = 1) and asphyxia due to oral hematoma (*n* = 1).

relapse and the other died of interstitial pneumonia. However, five of the seven (71%) undergoing allogeneic PBSCT after prior allogeneic HSCT died of infectious complications. The probability of PFS at 1 year in patients undergoing primary allogeneic PBSCT ($n = 33$) or allogeneic PBSCT after prior autologous PBSCT ($n = 10$) was 25.4 and 20.0%, respectively. The probability of OS at 1 year in patients undergoing primary allogeneic PBSCT or allogeneic PBSCT after prior autologous PBSCT was 26.0 and 20.0%, respectively. None of the patients undergoing allogeneic PBSCT after prior allogeneic HSCT survived ⩾196 days after transplant (Figure 1).

There was no significant difference in the incidence of TRM at day 100 post transplant between patients receiving unmanipulated PBSC ($n = 18$, 56%) and purified CD34+ cells ($n = 32$, 44%). The probability of PFS at 1 year in patients receiving unmanipulated PBSC or purified CD34+ cells was 22.2 and 17.6%, respectively ($P = 0.63$). The probability of OS at 1 year in patients receiving unmanipulated PBSC or purified CD34+ cells was 26.7 and 17.9%, respectively ($P = 0.93$; Figure 2).

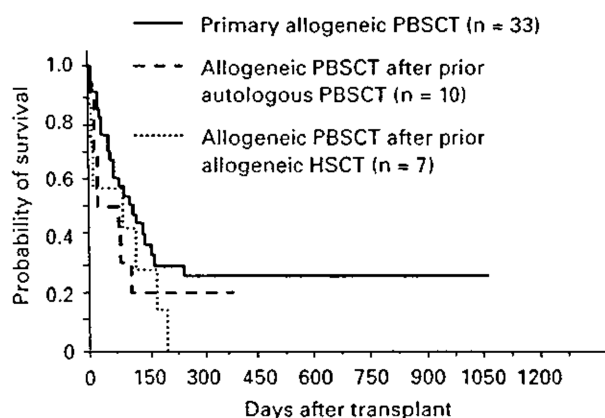

Figure 1 Probability of OS for patients undergoing primary allogeneic PBSCT, and allogeneic PBSCT after prior autologous PBSCT and allogeneic HSCT. The probability of OS at 1 year in patients undergoing primary allogeneic PBSCT or allogeneic PBSCT ($n = 33$, solid line) after prior autologous PBSCT ($n = 10$, dashed line) was 26.0 and 20.0%, respectively. None of the patients undergoing allogeneic PBSCT after prior allogeneic HSCT ($n = 7$, dotted line) survived ⩾196 days after transplant.

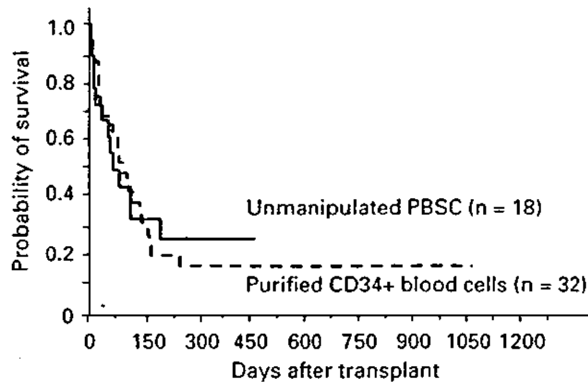

Figure 2 Probability of OS of patients receiving unmanipulated PBSC and purified CD34+ blood cells. The probability of OS at 1 year in patients receiving unmanipulated PBSC ($n=18$, solid line) or purified CD34+ blood cells ($n=32$, dashed line) was 26.7 and 17.9%, respectively ($P=0.93$).

There was no significant difference in the incidence of TRM at day 100 post transplant between patients receiving a conventional ($n=31$, 48%) and a reduced-intensity conditioning regimen ($n=19$, 47%). The probability of PFS at 1 year in patients receiving a conventional or a reduced-intensity conditioning regimen, was 26.3 and 16.2%, respectively ($P=0.86$). The probability of OS at 1 year in patients receiving a conventional or a reduced-intensity conditioning regimen was 26.3 and 17.0%, respectively ($P=0.77$).

Discussion

Our data support the notion that the present approach may be a possible option for a realistic alternative rescue for patients who lack an HLA-matched or a one-locus-mismatched related or unrelated donor, because we found that a significant proportion of patients with high-risk hematologic malignancies survived this procedure. As expected, we found that graft manipulation reduced the incidence of grades II–IV acute GVHD. However, there was no significant difference between the probabilities of PFS and OS at 1 year in patients receiving unmanipulated PBSC and purified CD34+ cells. We confirmed that transplant-related problems, particularly infectious complications, before day 100 post transplant were still major obstacles to the success of this therapy.

In the present analysis, we found that there were no significant differences in the time interval to neutrophil engraftment between patients receiving unmanipulated PBSC and purified CD34+ cells. On the other hand, we found that all graft failures (5%, 2/39) and rejections (8%, 3/37) were observed in those receiving purified CD34+ cells. We previously reported that in 13 Japanese children receiving purified CD34+ cells from a partially MMRD,[28] the observed rates of graft failure and rejection were 31% (4/13) and 22% (2/9), respectively. Kato *et al*[29] analyzed the clinical course in 135 young Japanese patients who underwent allogeneic HSCT with purified CD34+ cells from a haploidentical related donor, and found that graft failure occurred in 13% (13/103) of the patients with malignant diseases. Hence, the incidence of graft failure in the current study appears to be less than those in the previous Japanese reports. However, Aversa *et al*[30] reported that graft failure and rejection was 5% (2/43) and 0% (0/41), respectively. Recently, Redei *et al*[31] showed that all evaluable patients, who received $\geqslant 5\times10^6$ CD34+ blood cells purified by an Isolex system without cryopreservation, and were given a conditioning regimen including high-dose TBI, thiotepa, fludarabine and ATG without post transplant immunosuppressive treatment, established successful neutrophil engraftment. As we felt we were able to employ less immunosuppressive conditioning regimens to reduce transplant-related problems, we assumed that graft failure and rejection occurred at higher rates in our study than these two published studies. Additionally, in an attempt to reduce the risk of graft failure, various methods, including purification of blood cells to acquire very large numbers of CD34+ cells, follow-up DLI or T-cell add-back, and various types of anti-lymphocyte or thymocyte antibodies have been investigated,[27,32,33] but we could not find a benefit of employment of these well-established methods in our data. The explanation may again be that most patients were heavily pretreated prior to allogeneic PBSCT from two- or three-loci-MMRD . On the other hand, because all patients with graft failure and two of three of those with graft rejection had received purified CD34+ cells mismatched for HLA-C antigen in the HVG direction, matching for this locus may be an important factor to prevent graft failure and rejection when using purified CD34+ cells. The results of our study again highlight the notion that this approach with purified CD34+ cells should be conducted in a well-designed larger study, focusing on the prevention of graft failure and rejection.

As expected, in this study, the incidence of grades II–IV acute GVHD was significantly lower in patients receiving purified CD34+ cells (28%) compared with unmanipulated PBSC (64%). Similarly, several other studies reported that graft manipulation reduced the incidence of grades II–IV acute GVHD (range 0–21%).[29–31] As our patients received higher doses of CD3+ cells contained in the Isolex-purified CD34+ cells, or less immunosuppressive conditioning regimens with or without various kinds of GVHD prophylaxis, the incidence of grades II–IV acute GVHD in patients receiving purified CD34+ cells may be higher in our study than in other studies. Nonetheless, overall, PFS and OS did not differ significantly between patients receiving unmanipulated PBSC and purified CD34+ cells. This may have been due to the many transplant-related problems before day 100 post transplant observed with this approach. Ruggeri *et al*[34] showed that donor-versus-recipient natural killer (NK)-cell alloreactivity could protect patients against GVHD in allogeneic haploidentical HSCT. Procedures for purifying CD34+ cells have recently been introduced for allogeneic PBSCT from two- or three-loci-MMRD with the development of the CliniMACS and Isolex systems. Although further studies are needed to determine whether cell separation approaches can be applied to this approach, our data suggest that technological advances in cell separation of

PBSC allografts may influence the incidence of acute GVHD.

We could not find risk factors of RRT, VOD and TMA in pretransplant clinical status, such as PS and prior HSCT, in pretransplant treatment, such as conditioning regimens, or in post transplant treatment, such as GVHD prophylaxis and G-CSF use. Recently, new methods of transplantation, that is, nonmyeloablative pretransplant conditioning with or without ATG,[35] and improvements in supportive care in allogeneic HSCT, such as prevention and treatment of GVHD and infectious complications, have been introduced. However, none of the patients who underwent prior allogeneic HSCT survived ⩾196 days after transplantation. Five of the seven (71%) undergoing allogeneic PBSCT after prior allogeneic HSCT died of infectious complications. These results emphasize that this approach after prior allogeneic HSCT should be conducted in a well-designed study, focusing on the prevention of infectious complications, regardless of the application of conventional or reduced-intensity conditioning regimens.

We found that the major cause of death was infectious complications before 1 year post transplant (30%, 15/50), as has also been reported in several other studies (range 33–83%).[7,28–31] In our study, 20 patients (40%) suffered from bacterial sepsis before day 100 post transplant and 12 (24%) suffered from bacterial sepsis before achieving neutrophil engraftment. Thus, the effective prevention and treatment of bacterial infection occurring early after transplantation, especially before achieving neutrophil engraftment, may be of primary importance for the success of this approach. Although Volpi *et al*[36] have shown that G-CSF promotes T-helper (Th)-2 immune deviation, which, unlike Th1 responses, does not protect against fungi, G-CSF after transplant may be useful to reduce the incidence of infectious complications, particularly bacterial ones, because of achieving early neutrophil engraftment. On the other hand, delayed reconstitution of T cells may cause a high incidence of infectious complications, especially fungal and viral infections. Handgretinger *et al*[37] suggested that the transplantation of higher doses of purified CD34+ cells may hasten immune reconstitution. Thus, the number of transplanted CD34+ cells may be important to prevent fungal and viral infections. Although post transplant therapy, such as DLI or T-cell add-back, and reduced GVHD prophylaxis or reduced *in vivo* T-cell depletion may increase the incidence of GVHD, they may reduce the incidence of fungal and viral infections. They should be further investigated in future prospective trials. The incidence of infectious complications was high (75%) in our patients with a carry-over infection before the start of the conditioning regimen. Thus, we should therefore consider selection of patients very carefully and may exclude those with a carry-over infection, particularly MRSA and multidrug-resistant *P. aeruginosa*, from eligibility in any future study.

Our study was limited by the inevitable bias associated with a small population of patients treated at 20 different centers. Nevertheless, we found that a small but significant proportion of patients with high-risk hematologic malignancies survived this approach. In Japan, Kawano *et al*[28] previously reported that the probability of PFS and OS at 1 year was 30 and 40%, respectively, and Kato *et al*[29] reported that the probability of PFS at 67 months was 5.7%. Aversa *et al*[30] reported that the probability of OS at 18 months was 28% (including seven (16%) of 43 standard-risk patients). The probability of OS at 2 years in a study reported by Redei *et al*[31] was 25%. Although this approach is not significantly better in Japan than elsewhere in contrast to allogeneic HSCT from MUD, our data also support the notion that this modality may be a possible option as a realistic alternative rescue for patients who lack an HLA-matched or a one-locus-mismatched related or unrelated donor, regardless of whether they received unmanipulated PBSC or purified CD34+ cells, or the application of conventional or reduced-intensity conditioning regimens.

In conclusion, because only a small number of patients survived this procedure, future studies of allogeneic PBSCT with purified CD34+ cells should be conducted on the prevention of graft failure and rejection, and the observed high risk of treatment-related problems requires eligibility criteria for the selection of patients and the effective prevention and treatment of infectious complications occurring early after transplant. Our data suggested that patients undergoing prior allogeneic HSCT and those with a carry-over infection before the start of the conditioning regimen should also be excluded from this approach because their mortality from infectious complications was extremely high, regardless of whether they received unmanipulated PBSC or purified CD34+ cells, or the application of conventional or reduced-intensity conditioning regimens with or without various kinds of GVHD prophylaxis. The strategy for graft manipulation, and the application of conditioning regimens and GVHD prophylaxis should be further investigated in future prospective trials.

Acknowledgements

We wish to thank all the staff at the transplant centers who provided the patients with excellent clinical care. We would particularly like to thank Drs Tomohiko Kamimura, Kenjirou Kamezaki, Mika Kuroiwa, Haruko Kakumitsu, Tomoe Mizutani, Noriaki Kawano, Sung-Won Kim, Kentarou Minagawa, Naoki Hosen, Eui Ho Kim, Tomoki Masuda, Kazuhiro Ikegame, Akihiko Numata, Shouichi Inaba, Yoshihisa Kodera, Naoya Ochiai, Shin-ichi Fuchida, Satoko Morishima, Shigesaburo Miyakoshi, Kaichi Nishiwaki, Hiroshi Fujii, Takahisa Yamane, Kenji Kishi, Fumiaki Yoshiba, Hiroaki Ohnishi, Yoshikiyo Itoh, Kosei Matsue, Yoshinobu Kanda, Seishi Ogawa, Shigeru Chiba, Yasuhiko Miyazaki, Nobumasa Inoue, Harue Haruyama, Satoshi Murakami, Nobuhiko Uoshima, Mitsuru Tsudo, Hiroshi Akasaka, Jyun Ishikawa, Noriyuki Hirabayashi, Tatsuya Nakajyou, Hideyuki Takamatsu, Mitihiro Hidaka and Masato Masuda for participating in this research. We also thank Dr Naoko Kinukawa (Department of Medical Information Science, Kyushu University Hospital) for statistical analysis and Drs Takahiro Fukuda (Clinical Research Division, Fred Hutchinson Cancer Research Center), Masahiro Kami (Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital), Tetsuya Tanimoto (Medicine and Biosystemic Science, Kyushu University Graduate School) and Teruhisa Otsuka (Cancer Center, Kyushu University Hospital)

for helpful discussions. A complete list of the participating centers appears in the Appendix. This work was supported by the Ministry of Health, Labour and Welfare of Japan.

References

1 Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. *Nature* 2001; **411**: 385–389.
2 Thomas ED. Karnofsky Memorial Lecture. Marrow transplantation for malignant diseases. *J Clin Oncol* 1983; **1**: 517–531.
3 Rubinstein P, Carrier C, Scaradavou A *et al.* Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 1998; **339**: 1565–1577.
4 Laughlin MJ, Barker J, Bambach B *et al.* Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001; **344**: 1815–1822.
5 Beatty PG, Clift RA, Mickelson EM *et al.* Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med* 1985; **313**: 765–771.
6 Anasetti C, Amos D, Beatty PG *et al.* Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med* 1989; **320**: 197–204.
7 Henslee-Downey PJ, Abhyankar SH, Parrish RS *et al.* Use of partially mismatched related donors extends access to allogeneic marrow transplant. *Blood* 1997; **89**: 3864–3872.
8 Handgretinger R, Schumm M, Lang P *et al.* Transplantation of megadoses of purified haploidentical stem cells. *Ann NY Acad Sci* 1999; **872**: 351–362.
9 Ottinger H, Beelen D, Sayer H *et al.* Bone marrow transplantation from partially HLA matched related donors in adults with leukaemia: the experience at the University Hospital of Essen, Germany. *Br J Haematol* 1996; **92**: 913–921.
10 Aversa F, Tabilio A, Terenzi A *et al.* Successful engraftment of T cell-depleted haploidentical 'three-loci' incompatible transplants in leukemia patients by addition of recombinant human granulocyte blood progenitor cells to bone marrow inoculum. *Blood* 1994; **84**: 3948–3955.
11 Morishima Y, Morishita Y, Tanimoto M *et al.* Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings; possible role of genetic homogeneity. The Nagoya Bone Marrow Transplantation Group. *Blood* 1989; **74**: 2252–2256.
12 Oh H, Zhang M-J, Akiyama H *et al.* Comparison of graft-versus-host disease (GVHD) and survival in different ethnic populations: collaborative study by the Japan Adult Leukemia Study Group (JALSG) and the International Bone Marrow Transplant Registry (IBMTR). *Blood* 2002; **100**: 418 (Abstr. 1621).
13 Harada M, Nagafuji K, Fujisaki T *et al.* G-CSF-induced mobilization of peripheral blood stem cells from healthy adults for allogeneic transplantation. *J Haematother* 1996; **5**: 63–71.
14 Schumm M, Lang P, Taylor G *et al.* Isolation of highly purified autologous and allogeneic peripheral CD34+ cells using the CliniMACS device. *J Hematother* 1999; **8**: 209–218.
15 Martin-Henao GA, Picon M, Amill B *et al.* Isolation of CD34+ progenitor cells from peripheral blood by use of an automated immunomagnetic selection system: factors affecting the results. *Transfusion* 2000; **40**: 35–43.
16 Makino S, Harada M, Akashi K *et al.* A simplified method for cryopreservation of peripheral blood stem cells at −80 degrees C without rate-controlled freezing. *Bone Marrow Transplant* 1991; **8**: 239–244.
17 Przepiorka D, Weisdorf D, Martin P *et al.* Consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
18 Sullivan KM. Graft-versus-host disease. In: Forman SJ, Blume KG, Thomas ED (eds). *Bone Marrow Transplantation.* Blackwell Scientific Publications: Boston, MA, 1994, pp 339–362.
19 Bearman SI, Appelbaum FR, Buckner CD *et al.* Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol* 1988; **6**: 1562–1568.
20 McDonald GB, Hinds MS, Fisher LD *et al.* Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med* 1993; **15**: 255–267.
21 van der Plas RM, Schiphorst ME, Huizinga EG *et al.* von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. *Blood* 1999; **93**: 3798–3802.
22 Minagawa K, Yamasaki S, Ohno Y *et al.* Allogeneic stem cell transplantation (SCT) from a genotypical 2 or more loci mismatched related donor in the graft-versus-host direction: experience with adult Japanese patients. *Blood* 2002; **100**: 641a (Abstr. 2525).
23 Ottinger HD, Ferencik S, Beelen DW *et al.* Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. *Blood* 2003; **102**: 1131–1137.
24 Petersdorf EW, Gooley TA, Anasetti C *et al.* Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood* 1998; **92**: 3515–3520.
25 Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant* 2001; **28**: 909–915.
26 Klein JP, Rizzo JD, Zhang MJ, Keiding N. Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part 2: regression modeling. *Bone Marrow Transplant* 2001; **28**: 1001–1011.
27 Drobyski WR, Klein J, Flomenberg N *et al.* Superior survival associated with transplantation of matched unrelated versus one-antigen-mismatched unrelated or highly human leukocyte antigen-disparate haploidentical family donor marrow grafts for the treatment of hematologic malignancies: establishing a treatment algorithm for recipients of alternative donor grafts. *Blood* 2002; **99**: 806–814.
28 Kawano Y, Takaue Y, Watanabe A *et al.* Partially mismatched pediatric transplants with allogeneic CD34(+) blood cells from a related donor. *Blood* 1998; **92**: 3123–3130.
29 Kato S, Yabe H, Yasui M *et al.* Allogeneic hematopoietic transplantation of CD34+ selected cells from an HLA haploidentical related donor. A long-term follow-up of 135 patients and a comparison of stem cell source between the bone marrow and the peripheral blood. *Bone Marrow Transplant* 2000; **26**: 1281–1290.
30 Aversa F, Tabilio A, Velardi A *et al.* Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med* 1998; **339**: 1186–1193.
31 Redei I, Langston AA, Lonial S *et al.* Rapid hematopoietic engraftment following fractionated TBI conditioning and transplantation with CD34+ enriched hematopoietic progenitor cells from partially mismatched related donors. *Bone Marrow Transplant* 2002; **30**: 335–340.

32 Collins P, Watts M, Brocklesby M *et al.* Successful engraftment of haploidentical stem cell transplant for familial haemophagocytic lymphohistiocytosis using both bone marrow and peripheral blood stem cells. *Br J Haematol* 1997; **96**: 644–646.
33 Lewalle P, Triffet A, Delforge A *et al.* Donor lymphocyte infusions in adult haploidentical transplant: a dose finding study. *Bone Marrow Transplant* 2003; **31**: 39–44.
34 Ruggeri L, Capanni M, Urbani E *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097–2100.
35 Sykes M, Preffer F, McAfee S *et al.* Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myeloablative therapy and HLA-mismatched bone-marrow transplantation. *Lancet* 1999; **353**: 1755–1759.
36 Volpi I, Perruccio K, Tosti A *et al.* Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood* 2001; **97**: 2514–2521.
37 Handgretinger R, Lang P, Schumm M *et al.* Immunological aspects of haploidentical stem cell transplantation in children. *Ann NY Acad Sci* 2001; **938**: 340–358.

Appendix

The following transplant centers in Japan participated in this study: Kitakyushu Municipal Hospital, Hamanomachi Hospital, Toyama Prefectural Central Hospital, Harasanshin General Hospital, National Cancer Center Hospital, Osaka University Hospital, Kyushu University Hospital, Institute of Medical Science at the University of Tokyo, Japanese Red Cross Nagoya First Hospital, Kokura Memorial Hospital, Chiba University Hospital, Kyoto Prefectural University of Medicine Hospital, Meitetsu Hospital, Toranomon Hospital, Kashiwa Hospital at Jikei University, Kyoto First Red Cross Hospital, Osaka City University Hospital, Tokai University Hospital, Kagawa Medical University Hospital, Beppu National Hospital, Kameda General Hospital, University of Tokyo Hospital, Kansai University of Medicine Hospital, Osaka National Hospital, Social Insurance Kyoto Hospital, Matsushita Memorial Hospital, Osaka Red Cross Hospital, Nagoya Daini Red Cross Hospital, Kanazawa University Hospital, Kurobe City Hospital, Kumamoto National Hospital and Ryukyu University Hospital.

Peripheral blood circulating immature cell counts predict CD34+ cell yields in G-CSF-induced PBPC mobilization in healthy donors

Teruhiko Kozuka, Kazuma Ikeda, Takanori Teshima, Chikamasa Yoshida, Katsuji Shinagawa, Kensuke Kojima, Keitaro Matsuo, Akihiro Bessho, Kazutaka Sunami, Yasushi Hiramatsu, Yoshinobu Maeda, Toshio Noguchi, Kazuhiko Yamamoto, Nobuharu Fujii, Toshi Imai, Kinuyo Kaneda Kusumoto, Kozo Masuda, Katsuto Takenaka, Fumihiko Ishimaru, Kenji Niiya, Norio Koide, Mitsune Tanimoto, and Mine Harada

BACKGROUND: It has been previously reported that the number of circulating immature cells (CIC) in peripheral blood (PB) estimates the number of CD34+ cells collected in G-CSF plus chemotherapy-induced PBPC mobilization. The correlation of CIC counts in PB with CD34+ cell yield and its usefulness was evaluated in G-CSF-induced PBPC mobilization for healthy donors.

STUDY DESIGN AND METHODS: CIC counts in PB and CD34+ cell counts in the apheresis product from 122 collections were assessed, and the relationship between these two variables was evaluated with the Pearson rank correlation analysis, the chi-squared test, and the U-test.

RESULTS: CIC counts were correlated weakly with the number of CD34+ cells per L of blood processed in the apheresis product (Pearson rank correlation analysis; $r = 0.357$, $p < 0.0001$). When a level of 1.7×10^9 CICs per L was selected as a cutoff value, the sensitivity and specificity for collecting more than 20×10^6 CD34+ cells per L of blood processed were 63.6 and 77.5 percent, respectively.

CONCLUSION: The present study suggests that the number of CICs in PB may estimate the number of CD34+ cells collected. The data indicate that CIC counts above 1.7×10^9 per L can be used as a good predictor for PBPC collections containing more than 20×10^6 CD34+ cells per L of blood processed in a single apheresis procedure.

Allogeneic PBPCs are increasingly used as an alternative to marrow in the treatment of hematologic and nonhematologic malignancies. Rapid and sustained engraftment after PBPC transplantation depends on a sufficient number of HPCs infused. The number of CD34+ cells is widely used as an indicator for the engraftment potential of PBPCs transplanted. But, the universally accepted optimum number of precursors has not been established.[1-3] Recently, the accepted minimum recommended dose of CD34+ cells has been 4×10^6 per kg of recipient's body weight.[4,5] Many centers usually initiate apheresis procedures on Day 4 or 5, followed by G-CSF administration.

ABBREVIATIONS: CIC = circulating immature cells; HFR = high fluorescent reticulocyte; IMI = immature information; LFR = low fluorescent reticulocyte; MFR = intermediate fluorescent reticulocyte; PB = peripheral blood; ROC = receiver operating characteristic.

From the Second Department of Internal Medicine, Division of Blood Transfusion, Okayama University Medical School, Okayama; the Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Aichi; and Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.

Address reprint requests to: Teruhiko Kozuka, MD, Department of Internal Medicine, Ehime Prefectural Central Hospital, 83 Kasugamachi, Matsuyama 790-0024, Japan; e-mail: c-tkozuka@eph.pref.ehime.jp.

This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare of Japan; and Uehara Memorial Foundation.

Received for publication March 7, 2003; revision received November 14, 2003, and accepted November 18, 2003.

TRANSFUSION 2004;44:526-532.

However, the time to the maximum level of CD34+ cells in peripheral blood (PB) sometimes shows the interdonor differences.

We have previously reported that the number of circulating immature cells (CIC) in PB estimate the number of CD34+ cells collected in G-CSF plus chemotherapy-induced PBPC mobilization.[6,7] However, it is unknown whether these results would hold true in healthy individuals mobilized with G-CSF alone or in whom higher doses of G-CSF (5-10 µg/kg) were used. In the present study, we evaluated CIC counts as a simple and valid indicator of CD34+ cell collection in G-CSF-induced PBPC mobilization.

MATERIALS AND METHODS

Donors

Between November 1995 and June 2002, 57 healthy donors who were referred to the Okayama University Hospital (Okayama, Japan) for clinical indications of G-CSF-induced PBPC mobilization were consecutively entered into this study after informed consent was obtained. Median age of the healthy donors was 39 years old (range, 15-68). Gender of the healthy donors was 27 men and 30 women.

Mobilization regimens

Six donors (18 aphereses) received G-CSF (filgrastim, Kirin-Sankyo, Tokyo, Japan) at a dose of 5 µg per kg of body weight every 12 hours subcutaneously and 51 donors (104 aphereses) at a dose of 10 µg per kg of body weight every 24 hours subcutaneously. There was no difference in mobilization of CD34+ cells, CFU-GM, and BFU-E between the two G-CSF administration methods, and because the peak CD34+ cell mobilization was on Day 5,[8] apheresis procedures were started on Day 4 of G-CSF administration.

CIC quantities

Blood samples were obtained for WBC counts and WBC differentials each day after G-CSF administration. Conventional WBC differentials of 200 WBCs were performed on May-Giemsa-stained PB smears by one of our senior technologists who was not informed of this study. CIC counts, identified morphologically as myeloblasts, promyelocytes, myelocytes, metamyelocytes, and erythroblasts, were calculated by multiplying a percentage of CIC by each donor's WBC count. HPCs were also counted each day by an automated counter (SE-9000, Sysmex. Kobe, Japan). HPCs were detected in the IMI (immature information) channel. Detection of HPC was made possible by the combination of a special reagent system and direct current/radiofrequency biosensors. The lysis reagent contains detergents that are capable of lysing more mature WBCs because of their higher membrane lipid content, while HPCs remains relatively intact. Because various types of immature WBCs react differently to the reagent, they also occupy distinct areas on the bivariate matrix of the IMI scattergram.[9]

Collection of PBPCs

The method of PBPC collection has been reported previously.[7,10] Leukapheresis was performed with a continuous-flow blood cell separator (Spectra, COBE BCT, Lakewood, CO). Venous access was established by either peripheral vein or central venous catheter. Anticoagulant, ACD-A, was mixed at a ratio of 1 mL of anticoagulant to 12 to 17 mL of whole blood. The inlet flow rate was maintained at 60 to 100 mL per minute. The blood volume processed was 200 mL per kg of donor's body weight. The collection rate was adjusted between 1 and 2 mL per minute according to the rate of inlet flow. Daily leukapheresis was repeated usually two or three times to achieve the target of 4×10^6 CD34+ cells per kg of recipient's body weight.

Enumeration of CD34+ cells

The number of CD34+ cells collected and in PB was determined by a modified flow cytometric analysis technique.[7,10,11] A sample of 1×10^6 nucleated cells was stained with 10 µL of PE-conjugated mouse anti-CD34 MoAb (HPCA-2; Becton Dickinson, San Jose, CA) and 10 µL of peridinin chlorophyll protein-conjugated anti-CD45 MoAb (Becton Dickinson). Additional cells were stained with 10 µL of the anti-CD45 conjugate and 10 µL of PE-conjugated mouse IgG1 MoAb (Becton Dickinson) as an isotypic control. After incubation at 4°C for 15 minutes in a light-protected area, RBCs were lysed with lysing solution (FACS, Becton Dickinson) containing 0.83 percent ammonium chloride at room temperature for 10 minutes in a light-protected area, and washed twice with PBS containing 0.1 percent sodium azide. Analysis was performed on a fluorescence-activated cell sorter (FACScan, Becton Dickinson). Listmode data were analyzed with computer software (CELL Quest software, Becton Dickinson).

A gate was established to include all nucleated cells and to exclude PLTs and RBCs by use of the forward and 90° light scatter. A second gate was established to include only CD45+ cells with the side scatter. The number of events counted was 100,000. The number of bright CD34+ cells with the low side scatter was then determined. The percentage of CD34+ cells was calculated by subtracting the number of cells stained with the isotypic control from the number of cells stained with the anti-CD34 antibody and dividing by the number of nucleated cells counted in the first windows. WBC counts were obtained with an electronic cell counter (GENS, Beckman Coulter, Fullerton,

CA). The number of CD34+ cells was calculated from the total number of WBCs and the percentage of CD34+ cells.

Statistical analysis

Associations among various factors related to PBPC collections were analyzed with the Pearson rank correlation analysis, the chi-squared test, and the U-test. A p value less than 0.05 was defined to be significant. To define a cutoff CIC value for prediction of good mobilization, we applied the receiver operating characteristic (ROC) analysis. In the present study, good mobilization was defined as more than 20×10^6 CD34+ cells per L of processed blood volume.[12] Because, in the idealized situation of a donor undergoing apheresis for 200 mL per kg of donor's body weight, a CD34+ cell yield more than 20×10^6 per L of processed blood volume would be required to obtain the target cell dose of 4×10^6 per kg of body weight of the same body weight recipient in a single apheresis. A cutoff value was defined as round-off of the value that gave the best correct prediction for the defined good mobilization.

RESULTS

Cell counts in PB and apheresis products

PB cell counts and the number of CD34+ cells from all collections are shown in Table 1. A total of 122 PBPC leukapheresis procedures were performed in 57 donors. Median counts of WBCs and MNCs before apheresis were 39.8×10^9 per L (range, 19.2-90.7 $\times 10^9$/L) and 5.98×10^9 per L (range, 2.13-17.64 $\times 10^9$/L), respectively. The median CIC count in PB on the harvest day was 0.89×10^9 per L (range, 0-19.03 $\times 10^9$/L), respectively. The median number of MNCs collected was 312.0×10^8 (range, 102.0-875.0 $\times 10^8$). The median number of CD34+ cells collected per apheresis was 129.2×10^6 (range, 12.0-692.9 $\times 10^6$), and the median number of CD34+ cells per L of processed blood volume was 11.01×10^5 (range, 1.09-43.31 $\times 10^6$/L) in the apheresis product.

TABLE 1. Results of PBPC Collections

Total number of apheresis procedure	122
Number of apheresis procedures per patient	
1	4
2	34
3	19
Blood cell counts in PB on the day of harvest*	
WBCs count†	39.8 (19.2-90.7)
CICs count†	0.89 (0-19.03)
MNCs count†	5.98 (2.13-17.64)
Monocytes count†	2.01 (0-6.80)
Lymphocytes count†	3.72 (0.31-11.74)
PMNs count†	19.86 (8.21-44.12)
Bands count†	9.17 (2.50-40.82)
Platelets count†	177.0 (75.0-298.0)
Percentage of reticulocytes‡	1.5 (0.7-2.6)
Percentage of LFRs‡	86.0 (62.8-99.2)
Percentage of MFRs‡	13.0 (0.8-27.7)
Percentage of HFRs‡	0.8 (0-11.3)
HPCs†§	0.038 (0-0.417)
CD34+ cells in PB ($\times 10^6$/L)¶	23.94 (0-262.70)
Number of cells harvested	
MNCs ($\times 10^8$)	312.0 (102.8-875.0)
MNCs ($\times 10^8$/kg)	5.84 (1.64-13.97)
MNCs/processed blood volume ($\times 10^8$/L)	28.57 (9.27-67.50)
CD34 cells ($\times 10^6$)	129.2 (12.0-692.9)
CD34 cells ($\times 10^6$/kg)	2.32 (0.23-9.00)
CD34 cells/processed blood volume ($\times 10^6$/L)	11.01 (1.09-43.31)

* Shown is the median (range) for those patients who underwent G-CSF-induced mobilization regimens.
† Data reported are $\times 10^9$/L.
‡ The quantity of reticulocytes, LFRs, MFRs, and HFRs (%) was available for 119 collections in all 122 collections.
§ The quantity of HPCs was available for 21 collections in all 122 collections.
¶ The quantity of CD34+ cells in PB was available for 85 collections in all 122 collections. Abbreviations: LFR(s) = low fluorescent reticulocyte(s); MFR(s) = intermediate fluorescent reticulocyte(s); HRF(s) = high fluorescent reticulocyte(s); HPC(s) = hematopoietic progenitor cells(s).

Cell counts in PB and the number of CD34+ cells collected

Relationships between various PB cell counts before apheresis and the number of CD34+ cells collected per L of processed blood volume are shown in Fig. 1. CIC counts were correlated significantly with the number of CD34+ cells collected per L of processed blood volume (Pearson rank correlation analysis, $r = 0.357$, $p < 0.0001$). CIC levels in PB showed stronger correlation with the number of CD34+ cells collected per L of processed blood volume than WBC and MNC counts. Band form neutrophils (bands), reticulocyte, intermediate fluorescent reticulocyte (MFR) and high fluorescent reticulocyte (HFR) levels in PB also showed correlation with the number of CD34+ cells collected per L of processed blood volume. Low fluorescent reticulocyte (LFR) levels in PB showed inverted correlation with the number of CD34+ cells collected per L of processed blood volume. PMNs, monocyte, lymphocyte, PLT, and HPC levels in PB did not show correlation with the number of CD34+ cells collected per L of processed blood volume. CD34+ cells in PB showed the strongest correlation with the number of CD34+ cells collected per L of processed blood volume ($r = 0.529$, $p < 0.0001$).

Predictive value of CIC levels in PB before apheresis

Next, we evaluated levels of CIC, WBC, and CD34+ cells in PB as an indicator for estimating probability of obtaining more than 20×10^6 CD34+ cells per L of processed blood volume. A ROC curve was used to define the cutoff value and efficacy of these indicators. ROC curve is made up of

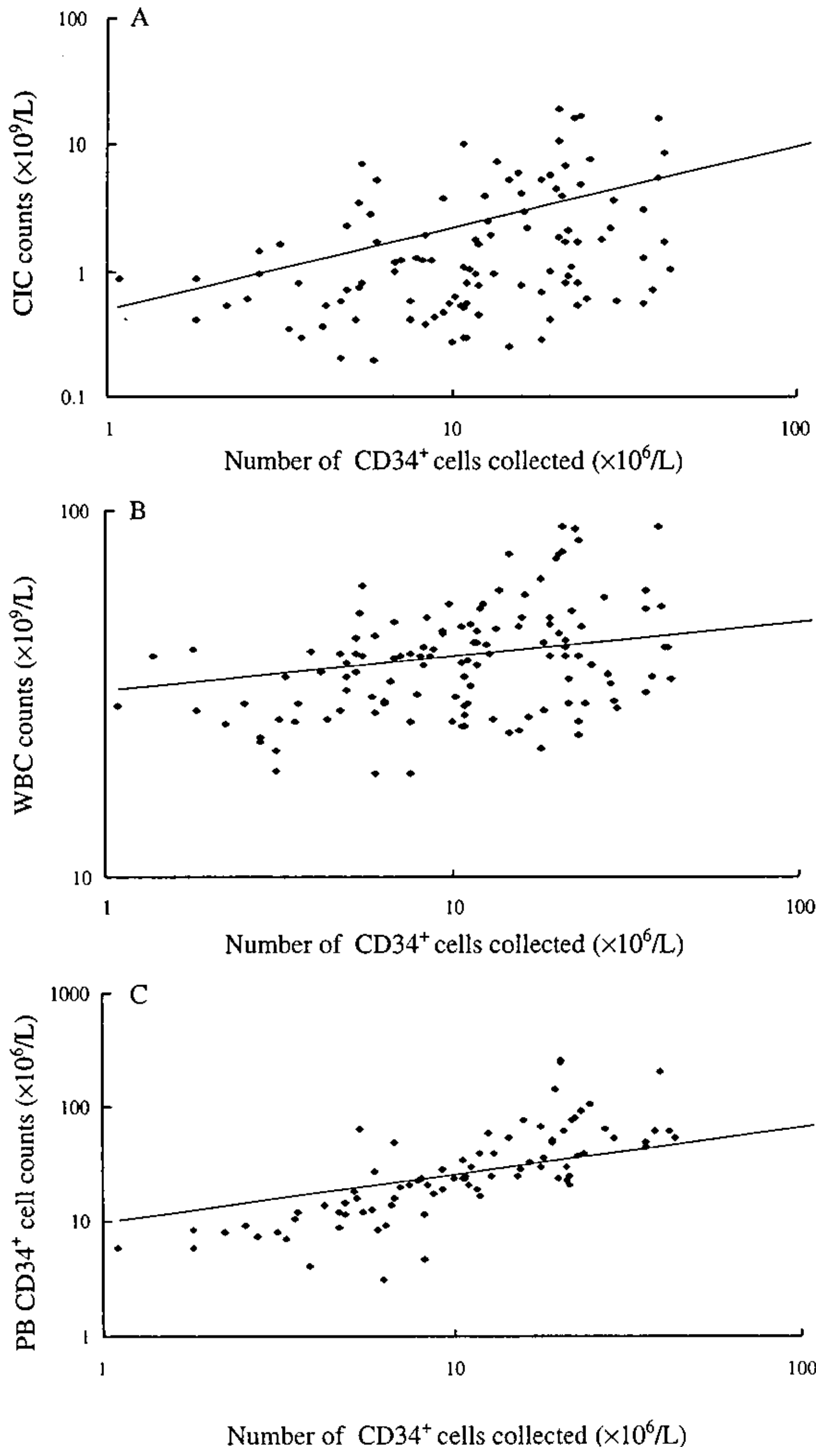

Fig. 1. Correlation of CIC counts (A), WBC counts (B), CD34+ cell counts (C) in PB on the harvest day (vertical axis), and the number of CD34+ cells collected (horizontal axis). A regression line is shown. (A) $r = 0.357$, $p < 0.0001$; (B) $r = 0.333$, $p < 0.00021$; (C) $r = 0.529$, $p < 0.0001$.

collection of plots representing a combination of sensitivity and specificity as a function of specific cutoff value. When the cutoff value is continuously changed from a very low value to a very high value, the plot moves from the top-right corner to the bottom-left corner along the curve. Generally, a cutoff value that gives the plot closest to the top-left corner is selected. Among these indicators, CD34+ cell counts in PB showed the highest sensitivity and specificity, and WBC counts did the lowest ones. Cutoff values of these indicators were 1.696×10^9 CICs per L, 42.4×10^9 WBCs per L, and 35.1×10^6 CD34+ cells per L in this analysis (Fig. 2). When the cutoff values of CIC, CD34+ cells in PB, and WBC counts were defined as 1.7×10^9 per L, 35×10^6 per L, and 42×10^9 per L, respectively, the sensitivity and specificity for obtaining more than 20×10^6 CD34+ cells per L of processed blood volume were 63.6 and 77.5 percent, 78.3 and 82.3 percent, and 54.5 and 66.3 percent, respectively (Table 2).

Other factors affecting the number of CD34+ cells

We evaluated levels of WBCs, MNCs, monocytes, lymphocytes, PMNs, bands, PLTs, reticulocytes, LFRs, MFRs, HFRs, HPCs, CD34+ cells in PB, MNCs in apheresis products, and MNCs per L of processed blood volume in apheresis products, as a predictor for estimation of collecting CD34+ cells of more than 20×10^6 per L of processed blood volume. Cutoff points were determined by the ROC curve for each of the variables (data not shown). Implications of each of the variables and its cutoff points were analyzed by the U-test (data not shown). Variables except lymphocytes, PMNs, PLTs, reticulocytes, and HPCs were significantly associated with the number of CD34+ cells collected. Also evaluated were age (>38 or <38 years old) and sex (men or women). The chi-squared test indicated that age less than 38 years old and men were significantly associated with collection of CD34+ cells at least 20×10^6 per L of processed blood volume ($p = 0.0288$ and $p = 0.0190$, respectively) (Table 3).

DISCUSSION

PBPC transplantation is now increasingly used as an alternative to marrow transplantation after high-dose cyto-

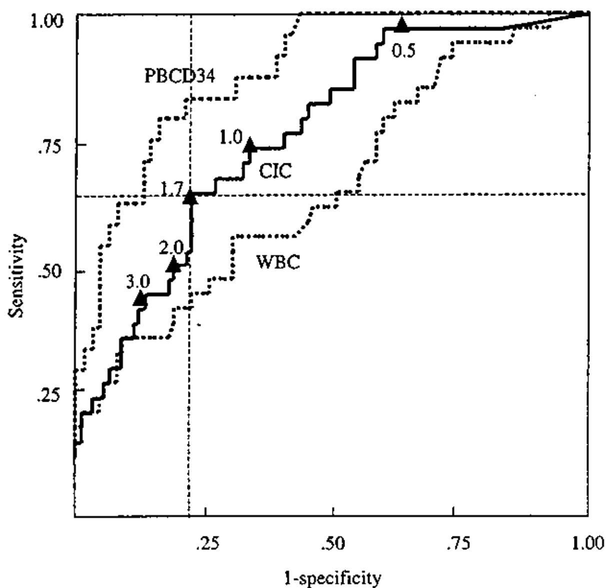

Fig. 2. ROC curve for CIC counts, WBC counts, and CD34+ cell counts in PB for obtaining good mobilization (more than 20 × 10^6 CD34+ cells/L of processed blood volume). Vertical and horizontal axes represent sensitivity and 1-specificity, respectively. The ROC curve is made up of collection of plots representing a combination of sensitivity and specificity as a function of specific cutoff value. When the cutoff value of each of the variables is continuously changed from a very low value to a very high value, the plot moves from the top-right corner to the bottom-left corner along the curve. Generally, a cutoff value that gives the plot closest to the top-left corner is selected, which corresponded to 1.696 × 10^9 CICs per L of processed blood volume in this analysis. "0.5," "1.0," "1.7," "2.0," and "3.0" indicate the plots representing a combination of sensitivity and specificity when the CIC cutoff value is defined as each value. CIC: sensitivity, 63.6 percent; specificity, 77.5 percent. PB CD34: sensitivity, 78.3 percent; specificity, 82.3 percent.

TABLE 2. Probability of Obtaining More Than 20 × 10^6 CD34⁺ Cells/L when the cutoff value of CIC, CD34⁺ cell in PB, and WBC are defined by ROC curve

	$<20 \times 10^6$ CD34⁺ cells/L	$>20 \times 10^6$ CD34⁺ cells/L
$<1.7 \times 10^9$ CIC/L	69 (77.53%)	12 (36.36%)
$>1.7 \times 10^9$ CIC/L	20 (22.47%)	21 (63.64%)
$<35 \times 10^6$ PB CD34⁺ cell/L*	51 (82.26%)	5 (21.74%)
$>35 \times 10^6$ PB CD34⁺ cell/L*	11 (17.74%)	18 (78.26%)
$<43 \times 10^9$ WBC/L	59 (66.29%)	15 (45.45%)
$>42 \times 10^9$ WBC/L	30 (33.71%)	18 (54.54%)

* The quantity of CD34⁺ cells in PB was available for 85 collections in all 122 collections.

TABLE 3. Distribution by Subject's Characteristics and CD34⁺ Cells Collected*

	CD34⁺ cells collected by an apheresis ($\times 10^6$/L)		
	<20	>20	p value
Number of donors	42	15	
Age (years)			
>38 vs. <38	25 (86)/17 (61)	4 (14)/11 (39)	0.0288
Sex			
Men vs. women	16 (59)/26 (87)	11 (41)/4 (13)	0.0190

* Shown are the distribution of subjects according to each parameters and the number of CD34⁺ cells collected (<20 or >20 $\times 10^6$/L) among the donors who underwent G-CSF-induced PBSC mobilization regimens. Values expressed as number of donors (%). All parameters were available for all 57 donors. The p values were obtained by the chi-squared test.

toxic therapy because of rapid hematologic recovery that gives rise to reduced toxicity, reduced requirement of antibiotics and blood transfusion, and shorter hospitalization. To collect a sufficient number of PBPCs for rapid and sustained engraftment, it is critical to optimize the harvesting condition.

It is relatively difficult to make a decision about when leukapheresis should be started and how many times it will be performed because the time to the maximum level of CD34+ cells in PB sometimes shows the interpatient difference. G-CSF begins to increase the donor's WBC count within 4 hours after the first dose is given,[13] but the increase in blood HPCs is delayed. Usually, the concentration of CD34+ cells in PB begins to increase and reaches a peak level on Day 3 and on Day 5 of G-CSF administration, respectively.[8] Although WBC counts remain elevated as long as G-CSF is given, the increase of HPCs is transient.[14] Therefore, many centers usually initiate apheresis procedures on Day 4 or 5. Some centers have reported that the majority of normal donors require only one apheresis for the collection of a target CD34+ cell dose of 4 × 10^6 per kg of recipient body weight.[15,16] However, a fraction of healthy donors do not provide effective mobilization, and procurement of the target CD34+ cell dose may require multiple procedures or may not be achieved despite daily apheresis.[15,16] In the present study, we have found that, for 16 percent of healthy donors, the CD34+ cell collection is insufficient to a target CD34+ cell dose of 4 × 10^6 per kg of recipient body weight even with aphereses for 3 days.

We previously reported that the number of CICs in PB estimate the number of CD34+ cells collected in G-CSF plus chemotherapy-induced PBPC mobilization.[6,7] In the present study, it was shown that the number of CICs is one of the predicting markers of the number of CD34+ cells collected and can be used for quickly estimating a would-be yield of CD34+ cells before apheresis, and predicting the number of apheresis in healthy individuals mobilized with G-CSF alone.

By analyzing sensitivity and specificity of the predictive value of CIC, we found that the number of CICs in PB was weakly correlated with the number of CD34+ cells

collected better than the WBC level and worse than CD34+ level in PB. Although correlation analysis between CICs and CD34+ cells per L of processed blood volume collected showed significance, careful interpretation is still required with referencing to 95-percent CI of correlation coefficients. Because the calculated coefficient of determinants, squared value of the correlation coefficient, which indicates the level of influence accounting for the variance of the change in CD34+ cells per L of processed blood volume, was not high enough. Although correlation coefficients between CD34+ level in PB and CD34+ cells per L collected were higher than those between CIC and CD34+ cells per L collected, the method of CIC counting is simple and inexpensive, and requires shorter time for analysis compared with flow cytometry. At least in Japan, and probably in other countries, some of the hospitals send their sample to commercial laboratories out of hospitals to enumerate CD34+ cells or cannot enumerate CD34+ cells at all for economical reason. For those hospitals, it would be an advantage to quickly estimate mobilization of CD34+ cells into blood, and, therefore, a yield of CD34+ cells before apheresis, and predict the number of apheresis procedures necessary to collect enough CD34+ cells for transplantation.

CIC counts in mobilization with G-CSF alone are less than those with G-CSF plus chemotherapy (median, 0.89 $\times 10^9$/L vs. 1.36 $\times 10^9$/L).[7] Generally, high CICs are observed in an early phase of rapid hematopoietic recovery after chemotherapy. Because there is no nadir phase induced by chemotherapy and the elevation of WBCs is slow in the setting of PBPC mobilization by G-CSF alone, CICs may not be increased as high as to predict the collected CD34+ cells in mobilization with G-CSF alone. Therefore, a correlation coefficient between CICs and CD34+ cells counts in mobilization with G-CSF alone may be less than that with G-CSF plus chemotherapy ($r = 0.357$ vs. 0.635).[7]

Recently, it has been reported that the counts of CD34+ cells and HPCs by an automated cell counter (SE-9000/9500, Sysmex, Long Grove, IL) can be used as a predictor of the number of CD34+ cells collected.[9,17] In the present study, HPCs in PB were not significantly associated with the number of CD34+ cells collected. This may be due to a small number of donors ($n = 21$) in this study.

By analyzing with the U-test, WBC, MNC, monocyte, Band, LFR, MFR, HFR, and CD34+ cell counts in PB, and MNC counts collected were associated with good PBPC mobilization. Rubia et al.[18] reported that donor's age, with a threshold of 38 years or more, is a factor that significantly affected CD34+ cell mobilization and collection in healthy donors. In the present study, it is also shown by the chi-squared test that the age and gender of donors, under 38 years old and men, respectively, were significantly associated with good PBPC mobilization. The result that men have more efficient mobilization than women is interesting. Although we do not know why men have better mobilization than women, this information would help in choosing donors.

There was no significant correlation between the number of infused CD34+ cells and engraftment outcomes (ANC to 500: $r = 0.179$, $p = 0.2341$; PLTs to 20000: $r = 0.251$, $p = 0.1246$), probably because most recipients received more than the critical CD34+ cell dose for rapid engraftment. There was no significant correlation between the number of infused CD34+ cells and Grade 2 to 4 acute GVHD ($r = 0.229$, $p = 0.1406$) either, probably reflecting the fact that the patients received several different pretransplant conditioning regimens and GVHD prophylaxis and that 10 of the donors were mismatched for one or two HLA loci. The correlation between the CD34+ cell dose and chronic GVHD was not evaluated because long-term follow-up data were not available.

We found that the number of CICs in PB showed a correlation with the number of CD34+ cells collected and thus could be used as a predictor for estimation of the timing of apheresis and the CD34+ cell yield. Although the correlation coefficients between CD34+ cells in PB and CD34+ cells collected was higher than CICs and CD34+ cells collected, the CIC counting has some benefits including its simplicity and inexpensiveness compared with CD34+ cell counting. In conclusion, our observations suggest that the CIC level ($>1.7 \times 10^9$/L) can be used for quickly estimating a would-be yield of CD34+ cells before apheresis and predicting the number of apheresis procedures.

ACKNOWLEDGMENTS

We thank Toshiyuki Watanabe, Hiroko Ishikura, Sakae Hongo, Hiroaki Ogo, and Naomi Asano, and the staff of the Apheresis Unit, Central Clinical Laboratory and Division of Blood Transfusion, Okayama University Medical School, for performing the blood counts and CD34+ cell counts.

REFERENCES

1. Blaise D, Kuentz M, Fortanier C, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Francaise de Greffe de Moelle. J Clin Oncol 2000;18:537-46.
2. Powles R, Mehta J, Kulkarni S, et al. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. Lancet 2000;355: 1231-7.
3. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001;344:175-81.
4. Siena S, Schiavo R, Pedrazzoli P, Carlo-Stella C. Therapeutic

relevance of CD34 cell dose in blood cell transplantation for cancer therapy. J Clin Oncol 2000;18:1360-77.

5. Miflin G, Russell NH, Hutchinson RM, et al. Allogeneic peripheral blood stem cell transplantation for haematological malignancies: an analysis of kinetics of engraftment and GVHD risk. Bone Marrow Transplant 1997;19:9-13.
6. Teshima T, Sunami K, Bessho A, et al. Circulating immature cell counts on the harvest day predict the yields of CD34+ cells collected after granulocyte colony-stimulating factor plus chemotherapy-induced mobilization of peripheral blood stem cell. Blood 1997;89:4660-1.
7. Kozuka T, Ikeda K, Teshima T, et al. Predictive value of circulating immature cell counts in peripheral blood for timing of peripheral blood progenitor cell collection after G-CSF plus chemotherapy-induced mobilization. Transfusion 2002;42:1514-22.
8. Yano T, Katayama Y, Sunami K, et al. G-CSF-induced mobilization of peripheral blood stem cells for allografting: comparative study of daily single versus divided dose of G-CSF. Int J Hematol 1997;66:169-78.
9. Takekawa K, Yamane T, Hino M, Tatsumi N. Determination of hematopoietic stem cells in peripheral blood by an automated hematology analyzer (SE-9000). Acta Haematol 1998;100:130-6.
10. Kozuka T, Ishimaru F, Fujii K, et al. Plasma stromal cell-derived factor-1 during granulocyte colony-stimulating factor-induced peripheral blood stem cell mobilization. Bone Marrow Transplant 2003;31:651-4.
11. Sutherland DR, Anderson L, Keeney M, et al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-26.
12. Anderlini P, Przepiorka D, Seong C, et al. Factors affecting mobilization of CD34+ cells in normal donors treated with filgrastim. Transfusion 1997;37:507-12.
13. Kerst JM, de Haas M, van der Schoot CE, et al. Recombinant granulocyte colony-stimulating factor administration to healthy volunteers. induction of immunophenotypically and functionally altered neutrophils via an effect on myeloid progenitor cells. Blood 1993;82:3265-72.
14. Stroncek DF, Clay ME, Petzoldt ML, et al. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. Transfusion 1996;36:601-10.
15. Anderlini P, Przepiorka D, Seong D, et al. Clinical toxicity and laboratory effects of granulocyte-colony-stimulating factor (filgrastim) mobilization and blood stem cell apheresis from normal donors, and analysis of charges for the procedures. Transfusion 1996;36:590-5.
16. Grigg AP, Roberts AW, Raunow H, et al. Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers. Blood 1995;86:4437-45.
17. Yu J, Leisenring W, Fritschle W, et al. Enumeration of HPC in mobilized peripheral blood with the Sysmex SE9500 predicts final CD34+ cell yield in the apheresis collection. Bone Marrow Transplant 2000;25:1157-64.
18. de la Rubia J, Arbona C, de Arriba F, et al. Analysis of factors associated with low peripheral blood progenitor cell collection in normal donors. Transfusion 2002;42:4-9.

Bone Marrow Transplantation (2004) 34, 909–914
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Cidofovir for treating adenoviral hemorrhagic cystitis in hematopoietic stem cell transplant recipients

K Nagafuji[1], K Aoki[1], H Henzan[1], K Kato[1], T Miyamoto[1], T Eto[2], Y Nagatoshi[3], T Ohba[4], K Obama[5], H Gondo[2] and M Harada[1]

[1]*Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan;* [2]*Department of Hematology, Hamanomachi General Hospital, Fukuoka, Japan;* [3]*Section of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan;* [4]*Department of Internal Medicine, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; and* [5]*Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan*

Summary:

Adenovirus (AdV) infection is an important cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. We treated 16 patients with AdV hemorrhagic cystitis (HC) following HSCT with cidofovir (CDV; 1 mg/kg/day, three times weekly for 3 weeks). Patients included 10 males and six females with a median age of 50 years (range 10–62). Two of the 16 patients were unevaluable because of early death from nonadenoviral causes. CDV therapy cleared AdV from urine in 12 of 14 patients (86%). Of 14 patients, 10 (71%) showed clinical improvements in HC. Among 14 patients, seven (50%) had avoided renal damage, the most important CDV toxicity. One patient previously treated with foscarnet for cytomegalovirus (CMV) required hemodialysis, and CDV treatment was discontinued. In another patient, CDV treatment was discontinued because of grade 2 nephrotoxicity. Four patients became positive for CMV antigenemia while being treated with CDV, and two developed herpes simplex virus (HSV) stomatitis while being treated with CDV. CDV proved effective in treating AdV HC in transplant patients. However, CDV at 1 mg/kg/day given three times weekly failed to prevent breakthrough infection with CMV and HSV in some patients.
Bone Marrow Transplantation (2004) 34, 909–914. doi:10.1038/sj.bmt.1704682
Published online 13 September 2004
Keywords: adenovirus; cidofovir; hemorrhagic cystitis; cytomegalovirus; herpes simplex virus

Adenovirus (AdV) infections including hemorrhagic cystitis (HC)[1–3] are emerging as life-threatening complications in hematopoietic stem cell transplant patients.[4] Reported occurrence rates of AdV infection complicating allogeneic hematopoietic stem cell transplantation (HSCT) vary from 5 to 21%,[1,5–8] and reported mortality rates have ranged from 7.7 to 38%.[6,9–11]

For treatment of AdV infection, reduction of immunosuppression[8] or infusion of donor lymphocytes[12] have been proposed. However, since AdV infections often occur in the presence of severe graft-versus-host disease (GVHD), immunotherapy may not be feasible. While specific anti-AdV therapy is therefore needed, no presently available drug has been proven to be effective, although some treatment success with ganciclovir (GCV),[13] vidarabine (AraA)[14] and ribavirin[15,16] have been reported. Unfortunately, these results could not be reproduced.[17] Cidofovir (CDV), a monophosphate nucleotide analogue of cytosine that inhibits viral DNA polymerase, demonstrates *in vitro* and *in vivo* activity against several viruses including herpesviruses, AdV, papilloma viruses, polyoma viruses, and poxvirus.[18] Several reports have described the effectiveness of CDV in post-transplant AdV disease.[19,20] The dose-limiting toxicity of intravenous CDV, when given at the recommended dose of 5 mg/kg once weekly, is nephrotoxicity.[18] Recently, a smaller, more frequent dose of CDV, 1 mg/kg/day three times weekly, demonstrated efficacy for treatment of post-transplant AdV infection.[20] In the present study, we have prospectively evaluated both toxicity and efficacy of CDV treatment for AdV HC in transplant patients. In all, 16 transplant patients were treated with CDV at a dose of 1 mg/kg, three times weekly for 3 weeks.

Materials and methods

Diagnosis of AdV HC

To exclude regimen-related HC, only patients who developed macroscopic hematuria with clinical signs of cystitis newly appearing *de novo* at least 10 days after HSCT and also had no tendency toward generalized bleeding or bacteriuria were considered to have HC.[21] According to previously reported criteria with minor modifications,[22] the severity of HC was graded as mild, sustained microscopic

Correspondence: Dr K Nagafuji, Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan;
E-mail: nagafuji@intmed1.med.kyushu-u.ac.jp
Received 27 April 2004; accepted 8 July 2004
Published online 13 September 2004

hematuria; moderate, gross hematuria and dysuria without clots; severe, gross hematuria and dysuria with clots. At the onset of HC, a urine specimen was obtained for viral culture and polymerase chain reaction (PCR). For rapid diagnosis, immunochromatography was performed (Adenocheck; Santen, Osaka, Japan). All patients underwent all the three diagnostic modalities (viral culture, PCR, and immunochromatography). When AdV was detected by one or more of these methods, a diagnosis of AdV HC was made.

Viral culture from urine

A 2 ml volume of urine was centrifuged overnight at 20 000 *g* and the sediment was added to culture of Hep-2 cells for up to 4 weeks. When a cytopathic effect of viral infection was observed, viral species were identified using monoclonal antibodies against AdV. Viral culture was carried out before the initiation of CDV treatment and 1 week after the last dose of CDV. The clearance of AdV was defined as the negative viral culture after treatment.

PCR of urine samples

After 2 ml of a urine sample was centrifuged at 15 000 *g* for 1 h at 4°C, the sediment was resuspended in 100 μl of PBS. DNA was purified using QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Next, 5 μl of purified DNA was subjected to PCR assay using a GeneAmp Kit and a GeneAmp PCR System 9600 (Perkin-Elmer, Boston, MA, USA). Primers used to screen for AdV infection were AD185S (5′-tccagcaacttcatgtccatgg-3′) and AD 185A (5′-tcgatgacgccgcggt-3). The size of the final products was confirmed by 3% agarose gel electrophoresis.[21]

Patient characteristics

In total, 16 patients were treated with CDV (10 males and six females with a median age of 50 years, ranging from 10 to 62). All patients had AdV HC. In all, 14 patients underwent allogeneic HSCT for acute myelogenous leukemia (AML) ($n=1$), acute lymphoblastic leukemia (ALL) ($n=2$), adult T-cell leukemia/lymphoma (ATL) ($n=2$), chronic myelogenous leukemia ($n=1$), myelodysplastic syndrome (MDS) ($n=1$), multiple myeloma (MM) ($n=2$), malignant lymphoma (ML) ($n=1$), and severe aplastic anemia (SAA) ($n=3$). Two other patients with systemic sclerosis underwent CD34+ cell autologous HSCT. Among allogeneic transplants, sources of stem cells were as follows: three from HLA-identical family donors, one from a DR-mismatch family donor, two from haplo-identical family donors, five from unrelated donors, and three from unrelated cord bloods. Patients No. 3 and no. 12 received antithymocyte globulin (ATG) as part of conditioning (Table 1).

Patient No. 2 died of cerebral infarction 5 days after initiation of CDV treatment, while patient No. 13 died from fungal pneumonia 11 days after initiation of CDV treatment. As these two patients with early death unrelated to AdV were excluded from analysis, 14 patients were evaluable. All patients received immunosuppressive therapy including cyclosporine, tacrolimus, and a steroid, as shown in Table 2. Serotypes of AdV isolated from urine were type 11 (11 patients), type 35 (one patient), or not determined (two patients). Onset of AdV HC ranged from 17 to 142 days after post transplantation (median, 37). Intervals between the onset of AdV HC and CDV treatment ranged from 0 to 56 days (median, 3). Two patients received AraA for treatment of AdV HC (Table 2).

CDV treatment

All of the patients gave their written informed consent in accordance with the requirements of the Institutional Review Board. The treatment regimen consisted of CDV, 1 mg/kg per day three times weekly for 3 weeks. Oral Probenecid (2 g) was given 3 h before CDV administration,

Table 1 Characteristics of patients

Patient no.	*Sex/age*	*Diagnosis*	*Transplant*	*Stem cell source*	*Use of ATG*	*Recipient CMV Ab*	*Donor CMV Ab*	*Recipient HSV Ab*
1	F/20	ALL/2CR	UBMT	DR mismatch	No	Positive	Negative	Positive
2	F/51	ATL/CR	CBT	B, DR mismatch	No	Positive	NE	Positive
3	M/32	SAA	UBMT	Match	Yes	Positive	Positive	Positive
4	F/54	SSc	autoPBSCT	CD34	No	Positive	NE	Positive
5	M/51	ML	alloPBSCT	Haplo-identical	No	Positive	Positive	Positive
6	F/41	ATL/Ref	alloPBSCT	Identical	No	Positive	Positive	Positive
7	F/52	MM	alloPBSCT	DR mismatch	No	Positive	Positive	Positive
8	M/47	ATL	UBMT	Match	No	Positive	Positive	Positive
9	M/17	SAA	BMT	Haplo	No	Positive	Positive	Positive
10	M/10	ALL/Ref	CBT	Three-loci mismatch	No	Positive	NE	Positive
11	M/36	CML/BC	alloPBSCT	Identical	No	Positive	Positive	Positive
12	M/45	SAA	UBMT	DR mismatch	Yes	Positive	Negative	Positive
13	M/62	MM	UBMT	B, DR mismatch	No	Positive	Negative	Positive
14	M/61	MDS	alloPBSCT	Identical	No	Positive	Positive	NE
15	M/50	AML/Ref	CBT	A, B mismatch	No	Positive	NE	NE
16	F/49	SSc	autoPBSCT	CD34	No	Positive	NE	Positive

ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; ATL = adult T-cell leukemia/lymphoma; SAA = severe aplastic anemia; ML = malignant lymphoma; CML = chronic myelogenous leukemia; CR = complete remission; Ref = refractory; BC = blastic crisis; MM = multiple myeloma; MDS = myelodysplastic syndrome; SSc = systemic sclerosis; BMT = bone marrow transplantation; UBMT = unrelated BMT; CBT = cord blood transplantation; PBSCT = peripheral blood stem cell transplantation; auto = autologous; allo = allogeneic; ATG = anti-thymocyte globulin; CMV = cytomegalovirus; HSV = herpes simplex virus; Ab = antibody; NE = not evaluated.

Table 2 characteristics of AdV disease

Patient no.	GVHD	Immunosuppressive at HC onset	Serotype of AdV	Viral study at the onset of HC culture/PCR/IC	Onset of HC (days from transplant)	Start of CDV administration (days from transplant)	Interval from onset to CDV treatment (days)	Prior therapy for HC
1	Grade II	FK506/PSL	11	+/+/+	40	59	19	AraA
3	No	Cs	11	+/+/+	29	30	1	No
4	NE	PSL	11	+/+/+	63	66	3	No
5	No	FK506/PSL	35	+/+/+	17	17	0	No
6	Grade II	Cs/PSL-FK506/ mPSL	ND	+/+/+	18	19	1	No
7	Grade II	Cs/PSL	ND	+/+/+	80	83	3	No
8	Grade II	PSL	11	+/+/+	53	109	56	AraA
9	No	FK506/PSL	11	+/+/+	25	25	0	No
10	Grade III	FK506	11	+/+/+	26	39	13	No
11	Chronic lung	FK506/PSL	11	+/+/+	142	149	7	No
12	No	FK506	11	+/+/+	34	34	0	No
14	Grade II	FK506/PSL	11	+/+/+	126	129	3	No
15	Grade II	Cs/PSL	11	+/+/+	50	64	14	No
16	NE	PSL	11	+/+/+	31	31	0	No

AdV = adenovirus; HC = hemorrhagic cystitis; CDV = cidofovir; IC = immunochromatography; NE = not evaluable; ND = not determined; GVHD = graft-versus-host disease; FK506 = tacrolimus; Cs = cyclosporine; PSL = prednisolone; AraA = vidaravin.

Table 3 Outcome of CDV treatment

Patient no.	Improvement of HC	Onset of effect (days)	Eradication of ADV from the urine*	Initial creat (mg/dl)	Max creat (mg/dl)	Final creat (mg/dl)	Renal toxicity (NCI-CTC)	Previous PFA treatment	Activation of herpesviruses during CDV treatment
1	Effective	6	Effective	1.38	1.38	1.02	1→1	No	None
3	No	—	Effective	0.54	0.83	1.02	0→0	No	None
4	Effective	7	Effective	0.76	1.02	0.59	0→1	No	None
5	No	—	Effective	1.2	2.38	1.79	1→2	No	CMV antigenemia, HSV stomatitis
6	Effective	13	Effective	0.54	0.59	0.59	0→0	No	CMV antigenemia
7	Effective	12	Effective	0.89	0.97	0.59	0→0	No	None
8	No	—	No	1.2	5.3	5.3	1→3	Yes	None
9	Effective	9	Effective	0.56	1.21	0.83	0→1	No	None
10	Effective	9	Effective	0.35	0.41	0.41	0→0	No	None
11	No	—	No	1.3	2.8	2.8	1→2	No	None
12	Effective	14	Effective	1	1.38	1.03	0→1	Yes	CMV antigenemia, HSV stomatitis
14	Effective	14	Effective	0.8	1.2	1	0→1	No	None
15	Effective	10	Effective	1.4	1.4	0.9	1→1	No	None
16	Effective	8	Effective	1.05	1.34	0.67	1→1	No	CMV antigenemia

*The eradication of AdV was defined by negative culture for AdV 1 week after the last dose of CDV.
Initial creat = serum creatine when starting CDV treatment; Max creat = maximal serum creatine during CDV treatment; Final creat = serum creatine upon completing CDV treatment; CDV = cidofovir; PFA = foscarnet; HC = hemorrhagic cystitis; AdV = adenovirus; CMV = cytomegalovirus; HSV = herpes simplex virus.

while 1 g was given 1 and 8 h afterward. Intravenous hydration with normal saline also was given. Patients were followed up for 2 months after the completion of CDV treatment.

Results

Outcome of CDV therapy

CDV therapy was successful in clearing AdV from the urine in 12 of 14 patients (86%), as defined by negative culture for AdV 1 week after the last dose of CDV. Of 14 patients, 10 (71%) showed clinical improvement in HC (Table 3). Median time to improvement of HC grade after CDV therapy was 9.5 days (range, 6–14; Table 3 and Figure 1). Patients No. 3 and No. 5 had persistent symptoms of HC despite eradication of AdV in the urine.

Toxicity

Serum creatinine concentration for all patients, at the time of initiation and termination of CDV treatment, as well as the maximum serum creatinine concentration during CDV treatment, are shown in Table 3. Renal toxicity was graded according to the Common Toxcity Criteria of National Cancer Institute (NCI-CTC Version 2.0; April 30, 1999). Among 14 patients, seven (50%) had no renal toxicity.

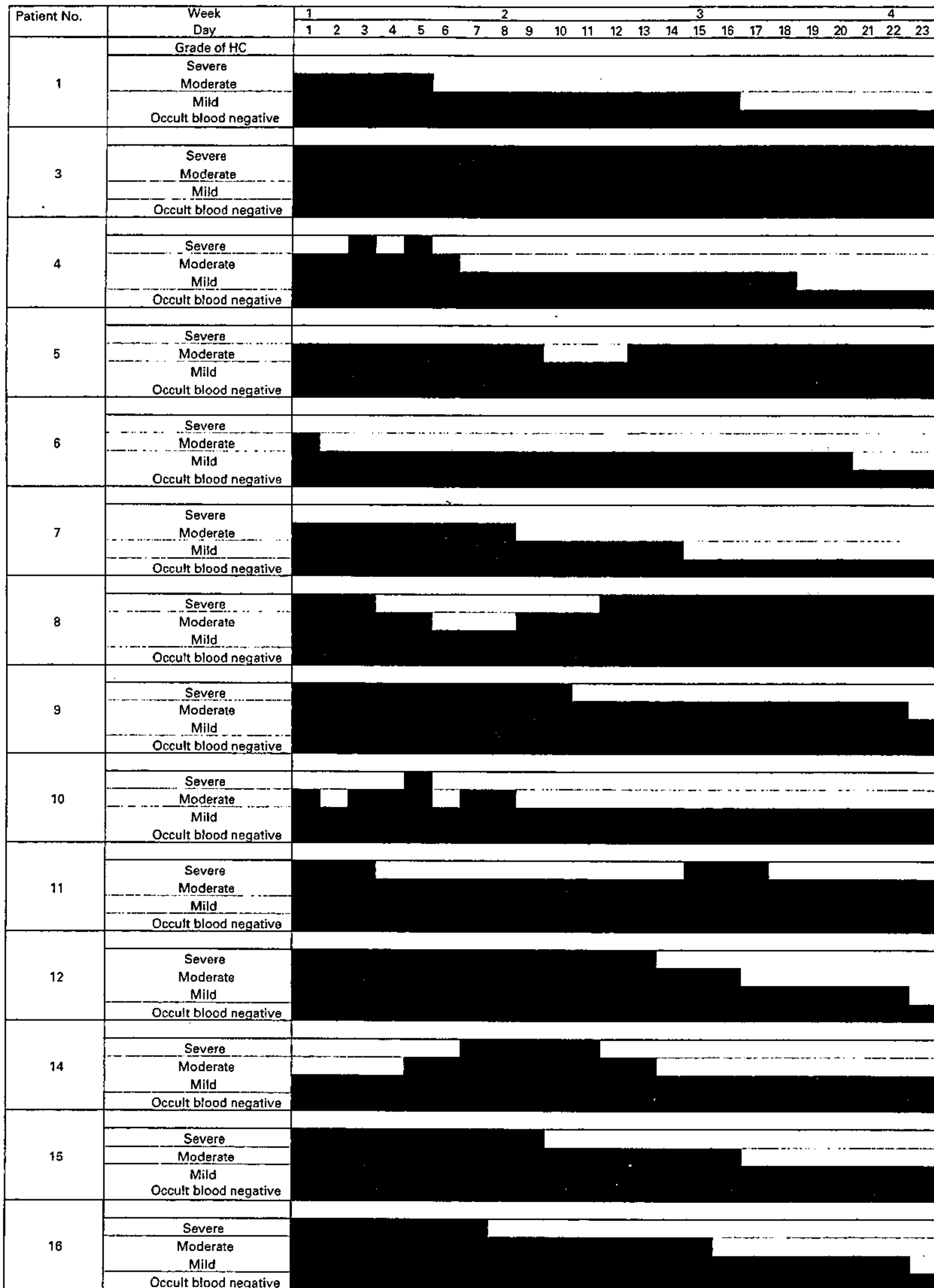

Figure 1 Clinical courses of 14 patients with adenoviral HC, who received cidofovir treatment.