In Vivo Alemtuzumab Enables Haploidentical Human Leukocyte Antigen-Mismatched Hematopoietic Stem-Cell Transplantation Without Ex Vivo Graft Manipulation

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Background. Alemtuzumab, a humanized monoclonal antibody directed against human CD52, has a strong lympholytic effect. This study evaluates the safety of unmanipulated peripheral blood stem-cell transplantation from two or three loci-mismatched related donors using alemtuzumab in vivo.

Methods. A total body irradiation-based regimen was used in young patients, whereas those 50 years or older received fludarabine-based conditioning. Alemtuzumab was added to these regimens by intravenous infusion at 0.2 mg/kg per day for 6 days (days −8 to −3).

Results. We treated 12 patients with a median age of 49.5 years. Eight patients demonstrated active disease, and four patients demonstrated acute leukemia in high-risk remission. All achieved neutrophil engraftment a median of 17.5 days after transplantation with complete donor-type chimerism. The cumulative incidence of grades III to IV acute graft-versus-host disease was only 9%. Infection-related deaths were not observed. CD3+/CD4+ and CD3+/CD8+ T cells were strongly suppressed within 2 months after transplantation, but recovered on day 90. Relapse was observed in five of eight patients who underwent transplantation for active disease, whereas none of the three patients who underwent transplantation in first remission had a relapse.

Conclusions. We conclude that in vivo alemtuzumab enables haploidentical hematopoietic stem-cell transplantation without ex vivo graft manipulation.

Keywords: Alemtuzumab, T-cell depletion, HLA mismatch, Allogeneic hematopoietic stem-cell transplantation, Graft-versus-host disease.

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Allogeneic hematopoietic stem-cell transplantation from a human leukocyte antigen (HLA)-identical sibling donor is an established treatment for hematologic malignancies. However, such a donor is available in only approximately 30% of patients in most developed countries (*1*, *2*). Therefore, alternative donor transplantation, including partially mismatched related donor transplantation, matched unrelated donor transplantation, and cord blood transplantation, has been investigated. Although transplantation from a one locus-mismatched related donor or a matched unrelated donor produces outcomes similar to those of transplantation from an HLA-identical sibling donor in high-risk patients (*3*), there is little chance of finding a one locus-mismatched related donor. In addition, it can sometimes be too time-consuming to coordinate a matched unrelated donor for patients with high-risk diseases. On the other hand, there is an excellent chance of identifying a family member who shares one haplotype with the patient and has two or three mismatched antigens in the second haplotype. Cord blood transplantation is also a possible alternative, but it is difficult to find a cord blood graft that contains enough nucleated cells for adult patients. Furthermore, it is impossible to obtain additional donor cells for immunotherapy after cord blood transplantation.

HLA incompatibility between the donor and recipient increases the risk of both graft rejection and severe graft-versus-host disease (GVHD). The outcome of two or three loci-mismatched transplantation without graft manipulation has been extremely poor (*3*, *4*), and thus it has been believed that ex vivo T-cell depletion from the graft is necessary to prevent severe GVHD. Although thorough T-cell depletion by CD34-positive cell selection has almost prevented GVHD (*5*), the incidences of graft rejection and infection increase after T-cell–depleted transplantation.

Campath-1 series of monoclonal antibodies is directed against human CD52, an antigen expressed on T, B, natural killer (NK), and dendritic cells, but not on hematopoietic stem cells (*6*, *7*). The original rat immunoglobulin (Ig)M and IgG monoclonal antibodies, Campath-1 M and Campath-1G, were used for ex vivo and in vivo T-cell depletion, respectively. The incidence of GVHD was significantly decreased by the use of these antibodies ex vivo only or both ex vivo and in vivo (*8–10*). Subsequently, Campath-1G was reshaped into a humanized form, alemtuzumab (Campath-1H), by genetic

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engineering (*11*). It has a longer terminal half-life (15–21 days) than Campath-1G (<1 day) (*12*). The addition of in vivo alemtuzumab to a conditioning regimen decreases graft rejection by depleting host T cells. In addition, it prevents GVHD because the alemtuzumab concentration is higher than that required to kill donor T cells at the time of graft infusion and remains at a potentially lympholytic level for approximately 2 months after transplantation (*13*). In fact, Mackinnon and coworkers showed that in vivo alemtuzumab has excellent prophylactic action against GVHD in a reduced-intensity conditioning regimen using fludarabine, melphalan, and alemtuzumab followed by stem-cell infusion mainly from HLA-matched donors (*14, 15*). However, there have been no reports on the application of in vivo alemtuzumab in two or three loci-mismatched transplantation. This study evaluates the safety of unmanipulated stem-cell transplantation from haploidentical two or three loci-mismatched related donors using alemtuzumab only in vivo.

PATIENTS AND METHODS

Patients

This study was approved by the ethical committee of the University of Tokyo Hospital, and all of the patients were seen and underwent transplantation at this hospital. Adult patients less than 65 years old who demonstrated high-risk acute leukemia, chemorefractory non-Hodgkin lymphoma, chronic myelogenous leukemia (CML) in blast crisis, myelodysplastic syndrome (MDS), or aplastic anemia with refractory severe neutropenia ($<500/mm^3$) were eligible for the study. The definition of high-risk acute leukemia included acute leukemia not in remission, in second or later remission, and in first remission with poor prognostic features such as positive Philadelphia chromosome (Ph^+), requiring more than two courses to achieve remission, and so on. Patients who had an available HLA-A/B/DR-matched or one locus-mismatched donor among family members were excluded. Patients who had an HLA-matched unrelated donor were also excluded unless the disease status precluded time-consuming donor coordination. Patients had to have a two or three loci-mismatched haploidentical related donor in good physical condition. Written informed consent was obtained from all patients and donors.

Stem-Cell Collection

Donors received granulocyte colony-stimulating factor at 200 $\mu g/m^2$ subcutaneously twice daily starting 3 days before the first collection of peripheral blood stem cells until the end of collection. Leukapheresis was performed daily until more than 5.0×10^6 CD34+ cells/kg of the recipient body weight were collected. Collected cells were then cryopreserved using standard techniques without ex vivo manipulation. The target cell dose was not achieved in three donors, but the minimum requirement dose had been set at 3.0×10^6 CD34+ cells/kg, and thus transplantation was performed using these grafts.

Conditioning Regimens

The conditioning regimen consisted of total body irradiation (TBI) at 2 Gy twice daily for 3 days (from days −7 to −5) and cyclophosphamide at 60 mg/kg per day for 2 days (from days −3 to −2). The dose of cyclophosphamide was decreased to 20 mg/kg per day for 2 days and etoposide at 40 mg/kg per day was added instead on day −4 in a patient with impaired cardiac function caused by anthracycline. For patients 50 years old or older, a non-TBI regimen consisting of fludarabine at 30 mg/kg per day for 6 days (days −8 to −3) and busulfan 1 mg/kg four times daily for 4 days (days −6 to −3) was applied. However, after we observed frequent relapse of lymphoid malignancies following this regimen, we added TBI at 2 Gy twice daily on day −1 and decreased the dose of busulfan to 4 mg/kg per day for 2 days (days −6 and −5) in the last two patients.

Alemtuzumab was added to these regimens at 0.2 mg/kg per day for 6 days (days −8 to −3). We adjusted the dose of alemtuzumab by body weight, because the body weight greatly differs among Japanese adult patients. The dose of daily alemtuzumab was determined by considering the total dose of alemtuzumab in previous studies (*14, 15*), the average body weight of white patients, and the daily dose of alemtuzumab in pediatric studies (*16*). To prevent acute infusion-related reactions to alemtuzumab, patients were pretreated with 1 mg/kg of methylprednisolone. Alemtuzumab was infused over 4 hr. On the first day of alemtuzumab infusion, 3 mg of alemtuzumab was infused over 2 hr and, after confirming that no severe infusion-related toxicities were observed, we infused the remaining alemtuzumab over the next 2 hr.

Other Transplantation Procedures

On day 0, the cryopreserved donor cells were thawed and infused. Prophylaxis against GVHD was performed with cyclosporine A (CsA) and short-term methotrexate. CsA was started on day −1 at a dose of 3 mg/kg per day by continuous infusion, and the dose was adjusted to maintain a blood concentration between 250 and 350 ng/mL. CsA was changed to an oral form when it could be tolerated by the patient. Methotrexate was administered at 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11. For patients without acute GVHD, we started to taper CsA from day 30 by 10% per week and discontinued CsA on day 100.

Prophylaxis against bacterial, fungal, and Pneumocystis carinii infection consisted of tosufloxacin, fluconazole, and sulfamethoxazole/trimethoprim. Some of the patients who had active or recent aspergillosis received antifungal prophylaxis with micafungin instead of fluconazole. As prophylaxis against herpes simplex virus infection, acyclovir was given 500 mg/day intravenously or 1,000 mg/day orally from days −7 to 35, followed by long-term, low-dose (200 mg/day) oral administration (*17*). Patients without myeloid malignancies received granulocyte colony-stimulating factor (filgrastim) at 300 μg/day by 3-hr infusion beginning on day 10 until the neutrophil count recovered to $500/mm^3$. Cytomegalovirus (CMV) antigenemia assay using C10/C11 antibody was performed at least once per week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides (*18*). Acute GVHD was graded as previously described (*19*). Patients who developed grades II to IV acute GVHD were treated with 1 mg/kg of intravenous methylprednisolone.

Host/donor cell chimerism after transplantation was analyzed by sex-chromosome FISH or the short tandem re-

TABLE 1. Patient characteristics

Median age	49.5 yr (range 27–60)	
Sex	Male 8/Female 4	
Diagnosis	ALL 5 (Ph^+ ALL 3), AML 2, MDS 2, CML-BC 1, NHL 2	
Disease status	Active disease	8
	High-risk remission	4
Comorbidities	Active/recent invasive aspergillosis	5
	Infective endocarditis, mitral valve replacement	1
	Anthracycline-induced cardiac failure	1
	Interstitial pneumonitis caused by radiation for breast cancer	1
	Diffuse lung infiltration of lymphoma	1
	Obstructive lung disease	1
	History of autologous transplantation	1
Donor	Sibling	4
	Son/daughter	6
	Uncle	1
	Cousin	1
No. of mismatched loci	Graft-versus-host direction 3 loci	7
	2 loci	5
	Host-versus-graft direction 3 loci	7
	2 loci	5
Conditioning regimen	Total body irradiation-based	6
	Fludarabine-based	6
Number of CD34+ cells in the graft	5.1×10^6 cells/kg (range 4.3–7.7)	
Number of CD3+ cells in the graft	2.6×10^8 cells/kg (range 1.8–7.1)	

ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; CML-BC, chronic myeloid leukemia-blast crisis; NHL, non-Hodgkin lymphoma.

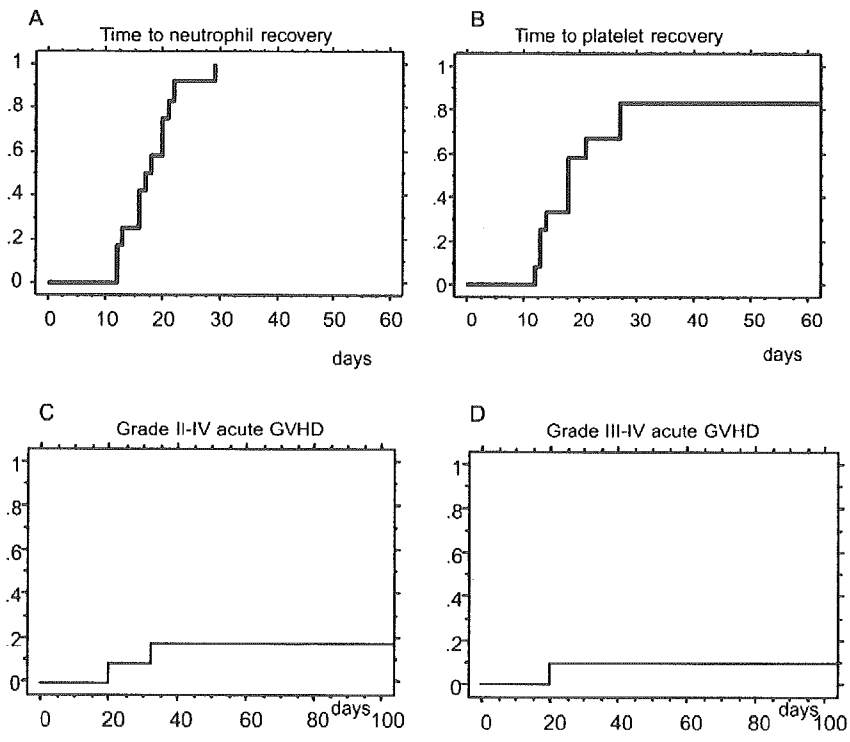

FIGURE 1. Days to neutrophil (A) and platelet (B) recovery and cumulative incidence of grades II to IV (C) and grades III and IV (D) acute GVHD after transplantation.

peat method monthly after transplantation (*20*). Immune reconstitution was evaluated by the quantification of CD3+/CD4+, CD3+/CD8+, CD3−/CD19+, and CD3−/CD56+ cells by flow cytometry. CMV-specific T-cell reconstitution was evaluated using fluorescent HLA-peptide tetramers in patients who were HLA-A*0201- or HLA-A*2402-positive

(*21*, *22*). As a functional assay, a phytohemagglutinin (PHA) stimulation test was performed as previously described (*23*).

Statistical Considerations

The primary endpoint of this study was the incidence of nonrelapse mortality within 100 days after transplantation. We defined success as the absence of early nonrelapse mortality and planned 7 and 9 patients in the first and second stages of this study, with target and lower success rates of 80% and 50% and alpha and beta errors of 10% and 10%, respectively (*24*). Nonrelapse mortality was observed in only one of the seven patients in the first stage, and thus the study was continued to the second stage. This was an interim analysis performed in February 2004. Overall survival and the incidences of GVHD and CMV reactivation were calculated using the Kaplan-Meier method. The data were compared with those who underwent allogeneic hematopoietic stem-cell transplantation from an HLA-identical sibling donor or a matched unrelated donor in the same period. Overall survival and the incidence of CMV reactivation were compared using the log-rank test. The recovery of immunologic parameters was compared using the Mann-Whitney *U* test.

RESULTS

Characteristics of the Patients

Twelve patients were included in the study (Table 1). There were eight males and four females with a median age of 49.5 years (range 27–60 years). The underlying disease was acute lymphoblastic leukemia (ALL) in five patients, acute myeloblastic leukemia in two patients, MDS in two patients, CML in blast crisis in one patient, and non-Hodgkin lymphoma in two patients. Eight patients demonstrated active disease at transplantation. The other four patients underwent transplantation for ALL in remission. Of these, two demonstrated Ph^+ ALL in first remission, one demonstrated ALL in second remission, and one demonstrated ALL in first remission and required more than 3 months to achieve remission. Most patients demonstrated comorbidities before transplantation including recent or active invasive aspergillosis in five, anthracycline-induced cardiac failure, interstitial pneumonitis caused by radiation for breast cancer, obstructive lung disease, and so on. Six patients who were more than 50 years old received a fludarabine-based regimen, whereas the other six received a TBI-based regimen.

Recovery of Donor Cells

The median number of CD34+ and CD3+ cells in the graft was 5.1×10^6 cells/kg (range 4.3–7.7) and 2.6×10^8 cells/kg (range 1.8–7.1), respectively. The median duration to the neutrophil recovery greater than 500/mm^3 and platelet recovery greater than 20,000/mm^3 without transfusion was 17.5 days (range 12–29 days) and 16 days (range 12–27 days), respectively (Fig. 1A and B). Complete donor-type chimerism was achieved on day 28 in all patients and was sustained thereafter, except for one patient who underwent transplantation for MDS (chronic myelomonocytic leukemia) using a fludarabine-based regimen and developed mixed chimerism (8.5% host cells) on day 60, and then relapsed with acute myeloblastic leukemia on day 90.

Graft-Versus-Host Disease

Grades II to IV acute GVHD was observed in two patients. One of the two patients developed grade II acute GVHD of the gut on day 32, which responded to methylprednisolone. The other patient developed grade III acute GVHD of the skin and gut on day 20, which was refractory to steroids, and eventually died of thrombotic microangiopathy on day 66. This patient received a three loci-mismatched graft from a cousin. He developed early hemorrhagic cystitis followed by postrenal azotemia and could not receive CsA at a therapeutic concentration. The cumulative incidence of grades II to IV and III to IV acute GVHD was 18% and 9%, respectively (Fig. 1C and D).

Of the eight evaluable patients who survived more than 100 days after transplantation, limited chronic GVHD that did not require treatment was observed in two patients. Notably, all five patients who are alive more than 100 days after transplantation as of this analysis are free from immunosuppressants.

Infectious Complications

Of the five patients who had recent or active invasive pulmonary aspergillosis before transplantation, one had a recurrence of aspergillosis during the neutropenic period after

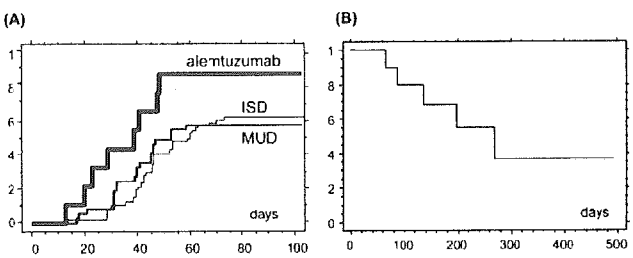

FIGURE 2. Cumulative incidence of cytomegalovirus reactivation detected by antigenemia assay, grouped according to the donor type (A). Overall survival of all patients (B). ISD, human leukocyte antigen (HLA)-identical sibling donors; MUD, matched unrelated donors.

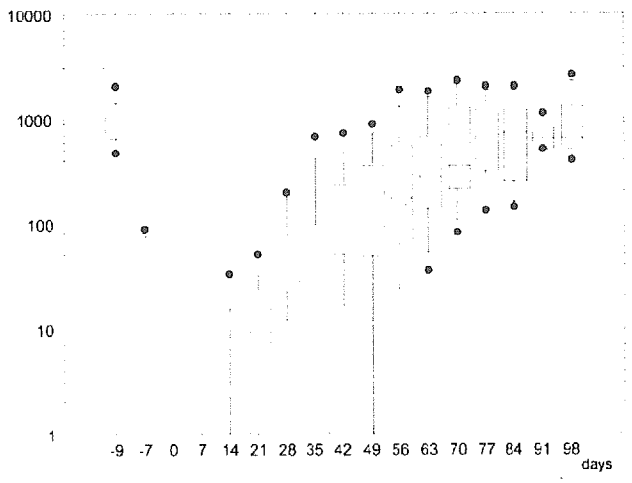

FIGURE 3. Recovery of peripheral blood lymphocytes after transplantation; 10, 25, 50, 75, and 90 percentile values (*box-and-whisker plot*). Outliers (*dots*).

transplantation, which was improved with neutrophil recovery. Otherwise, severe bacterial or fungal infection was not observed throughout the entire period after transplantation.

Of the 11 patients who were seropositive for CMV or who had a donor who was seropositive for CMV before transplantation, CMV reactivation was detected in 10 by antigenemia assay. The incidence of CMV reactivation was significantly higher than that after transplantation from an HLA-identical sibling donor or a matched unrelated donor (P=0.032, Fig. 2A). However, there was no death or severe disease related to CMV infection. Two patients developed asymptomatic CMV retinitis on days 149 and 160, respectively, and another patient developed hemorrhagic cystitis with CMV viruria on day 45, all of which were successfully treated with ganciclovir.

Relapse, Nonrelapse Mortality, and Survival

As a primary endpoint of the study, early nonrelapse mortality before day 100 was observed in one patient, who died of thrombotic microangiopathy and gut hemorrhage on day 66. Nonrelapse mortality was observed in another patient, who died of worsening of interstitial pneumonitis on day 197. This patient had received 60 Gy of local radiation to the right upper lung lobe for breast cancer and had already had local interstitial pneumonitis before transplantation. Six patients had a relapse of the underlying hematologic malignancy at a median of 111 days (range 49–223 days) after transplantation, and five of these had active disease before transplantation. Three of them died, and two are alive with disease. The remaining patient, who had undergone transplantation for ALL in second remission, received donor lymphocyte infusion after relapse of ALL and is alive in remission. None of the three patients who underwent transplantation for acute leukemia in first remission have relapsed thus far. Of these, two patients who had Ph^+ ALL were in molecular remission after transplantation. Overall survival is shown in Figure 2B.

Immune Reconstitution

The peripheral lymphocyte count dramatically decreased on the day after the first infusion of alemtuzumab and then gradually increased after day 28 (Fig. 3). Immune recon-

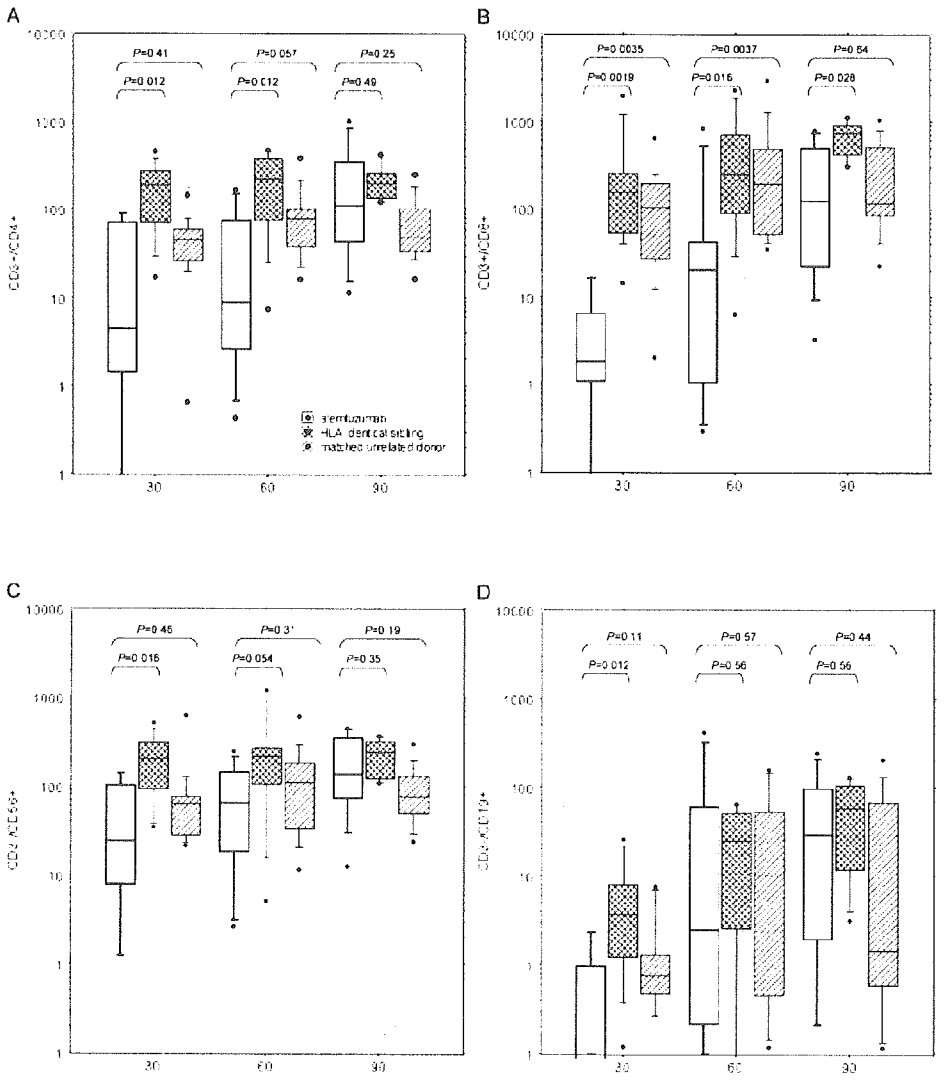

FIGURE 4. Recovery of CD3+/CD4+ (A), CD3+/CD8+ (B), CD3−/CD56+ (C), and CD3−/CD19(+) (D) cells on days 30, 60, and 90 after transplantation, grouped by the donor type; 10, 25, 50, 75, and 90 percentile values (*box-and-whisker plot*). Outliers (*dots*).

TABLE 2. Summary of outcome

Complications		
Infections	Possible invasive pulmonary aspergillosis	1
	Cytomegalovirus reactivation	10
	Cytomegalovirus retinitis	2
	Adenovirus hemorrhagic cystitis	2
Acute GVHD	0	6
	I	4
	II	1
	III	1
	IV	0
Chronic GVHD	None	6
	Limited	2
	Extensive	0
	Not evaluable	4
Outcome		
Status before transplantation	Current status	
Remission 4	Alive in remission	3
	Died in remission	1 (radiation pneumonitis)
Active disease 8	Alive in remission	2
	Alive after relapse	2
	Died after relapse	3
	Died in remission	1 (thrombotic microangiopathy)

GVHD, graft-versus-host disease.

stitution after transplantation was evaluated in greater detail by the quantification of CD3+/CD4+, CD3+/CD8+, CD3−/CD19+, and CD3−/CD56+ cells, and compared with that after transplantation from an HLA-identical sibling donor or a matched unrelated donor (Fig. 4). The numbers of CD3+/CD4+ and CD3+/CD8+ cells were significantly lower than those after transplantation from an HLA-identical sibling donor or a matched unrelated donor during the first 2 months after transplantation. However, the numbers of CD3+/CD4+ and CD3+/CD8+ cells caught up with those after matched unrelated transplantation on day 90. CD3−/CD56+ and CD3−/CD19+ cells recovered earlier than T cells.

CMV-specific T-cell recovery was evaluated by tetramer assay in six patients who had HLA-A*0201 or HLA-A*2402. CMV-specific cytotoxic T lymphocytes were detected on day 90 after transplantation in two patients, at 0.03% and 0.25% of CD8+ T cells, respectively. Both patients had CMV reactivation before the detection of CMV-specific cytotoxic T lymphocytes. As a functional assay, a PHA stimulation test was performed using peripheral lymphocytes in three patients on days 120, 377, and 509, respectively, after transplantation. The stimulation index was 415.2 and 391.0, respectively, in the two patients who were tested more than 1 year after transplantation. Considering that the 95% confidence interval for the stimulation index in the normal population is 74 and 508, peripheral T cells in these patients have a normal proliferative response to PHA stimulation. Although the stimulation index was only 10.7 in the patient who was tested on day 120, at that time only 6% of the peripheral lymphocytes were T cells, and thus the response was within the normal range when corrected for the percentage of T cells.

DISCUSSION

We evaluated the safety of haploidentical peripheral blood stem-cell transplantation from a two or three loci-mismatched family member using in vivo alemtuzumab. There was no graft rejection, and the incidence of grades III to IV acute GVHD was only 9%, almost equivalent to that after transplantation from an HLA-matched sibling donor (*3*). The median age of the 12 patients was 49.5 years, and 9 and 6 patients were older than 40 and 50 years, respectively. Thus, the patients in this study were much older than those in previous reports on haploidentical stem-cell transplantation (*5*, *25*, *26*). Nevertheless, nonrelapse mortality was observed in only two patients.

A major concern with this strategy was infectious complications caused by prolonged immunosuppression. In fact, CD3+/CD4+ and CD3+/CD8+ T cells were strongly suppressed within 2 months after transplantation, which was reflected by the high incidence of CMV reactivation. However, this did not translate into the development of severe infections. None of the patients died of infectious causes. Another concern was relapse after transplantation. Five of the eight patients who underwent transplantation for active disease had a relapse, whereas this was not seen in any of the three patients who underwent transplantation in first remission. Therefore, the dose of alemtuzumab is appropriate for patients with early disease, whereas it may be better to reduce

the dose of alemtuzumab for patients with active disease, considering the low incidence of severe GVHD in this study.

The transplantation procedure was simplified and the cost was reduced by the omission of ex vivo graft manipulation. Antithymocyte globulin (ATG) could also be used for in vivo T-cell depletion. However, considering the results of HLA-matched transplantation, the prophylactic effect of alemtuzumab against GVHD seemed to be much stronger than that of ATG (*14, 15, 20, 27*). The unique pharmacokinetic profile, in which a lympholytic concentration remains for approximately 2 months after transplantation, may contribute to the potent effect against GVHD (*13*). Depletion of host dendritic cells, which also express CD52, could be another mechanism to prevent GVHD, because host antigen-presenting cells have been shown to be important for the development of GVHD in mouse models (*28*). Alemtuzumab may be more appropriate for clinical use than ATG, because alemtuzumab is a recombinant monoclonal antibody with a consistent quality, whereas lot-to-lot variability of ATG cannot be avoided, because ATG is prepared by immunizing horses or rabbits with human lymphoid cells. Another advantage of alemtuzumab is that it kills not only T cells but also B cells, and thus there may be a lower risk of posttransplant lymphoproliferative disorders. A possible disadvantage of alemtuzumab is that alemtuzumab may kill NK cells, which may be important for a graft-versus-leukemia/lymphoma effect (*29*). However, the recovery of NK cells was observed early after transplantation in this study and was equivalent to that after matched unrelated donor transplantation. As further evidence that the lympholytic effect of alemtuzumab on NK cells is weaker than that on T cells, alemtuzumab "in the bag" resulted in the 99.8% and 94% depletion of CD4+ and CD8+ T cells, respectively, whereas 30% of NK cells were conserved in the graft (*30*).

CONCLUSION

Unmanipulated hematopoietic stem-cell transplantation was safely performed from a two or three loci-mismatched family member using in vivo alemtuzumab. This novel therapeutic approach could be applied to patients aged more than 50 years without the need for an HLA-matched donor or any specific devices. [AU: Please cite Table 2 in text.]

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Effect of Conditioning Regimen on the Outcome of Bone Marrow Transplantation from an Unrelated Donor

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ABSTRACT

Little information is available regarding the effect of the conditioning regimen on the outcome of bone marrow transplantation (BMT) from an unrelated donor. Therefore, we retrospectively compared the outcome after a cyclophosphamide/total body irradiation (Cy-TBI) regimen, an intensified Cy-TBI regimen (Cy-TBI$^+$), a busulfan and cyclophosphamide (Bu-Cy) regimen, and a Bu-Cy regimen with total lymphoid irradiation (Bu-Cy-TLI). Clinical data of 1875 adult patients who underwent unmanipulated unrelated BMT for leukemia or myelodysplastic syndrome by using 1 of the 4 regimens between 1993 and 2002 were extracted from the database of the Japan Marrow Donor Program. The effect of the conditioning regimen was adjusted for other independent significant factors by multivariate analyses. The Cy-TBI regimen was significantly better than the Bu-Cy regimen with regard to the incidence of engraftment failure (odds ratio, 2.49; $P = .046$) and overall survival (relative risk [RR], 1.31; $P = .050$). The Bu-Cy-TLI regimen decreased relapse (RR, 0.13; $P = .039$) but increased nonrelapse mortality (RR, 1.89; $P = .0061$). The Cy-TBI$^+$ regimen resulted in increased nonrelapse mortality (RR, 1.48; $P = .0003$) and inferior survival (RR, 1.45; $P < .0001$). The results of this retrospective study suggested that the Cy-TBI regimen was superior to other regimens in unrelated BMT.

KEY WORDS

Conditioning regimen • Unrelated bone marrow transplantation • Cyclophosphamide • Busulfan • Total body irradiation

INTRODUCTION

The conditioning regimen before allogeneic hematopoietic stem cell transplantation (HSCT) is intended to eradicate tumor cells and to promote immunosuppression to prevent graft rejection. Successful bone marrow transplantation (BMT) with a combination of cyclophosphamide (Cy) and total body irradiation (TBI) was reported in the 1970s [1,2]. TBI is effective against a variety of malignancies without sanctuary sites, such as the central nervous system and testicles. However, there has been concern regarding long-term sequelae, including cataracts, second malignancies, and development problems in children. Thus, non-TBI regimens have been investigated by substituting busulfan (Bu) for TBI [3,4]. Accordingly, the

Cy-TBI and Bu-Cy regimens have been regarded as the standard conditioning regimens since the 1980s.

Four randomized controlled trials have been performed to compare the Cy-TBI and Bu-Cy regimens in HSCT from an HLA-identical sibling donor, but they gave conflicting results with regard to both survival and toxicities [5-8]. Therefore, Hartman et al. [9] conducted a meta-analysis of these randomized controlled trials and 1 other trial that compared the etoposide/TBI regimen and the Bu-Cy regimen. They showed a significantly lower incidence of hepatic veno-occlusive disease (VOD) after the TBI-based regimen than the Bu-Cy regimen and a trend toward better survival after the TBI-based regimen ($P = .09$). Recently, Socié et al. [10] updated the 4 randomized controlled trials that compared the Cy-TBI and Bu-Cy regimens, with a mean follow-up for surviving patients of >7 years. Although the Cy-TBI and Bu-Cy regimens were associated with similar survival in patients with chronic myelocytic leukemia (CML), a nonsignificant ($P = .068$) 10% lower survival rate was observed after the Bu-Cy regimen in patients with acute myeloblastic leukemia (AML).

The feasibility of the Bu-Cy regimen in unrelated HSCT has been shown in several studies, but the incidence of engraftment failure ranged up to 12% [11,12]. The number of patients in these studies was small, and there has been no randomized controlled trial to compare conditioning regimens in unrelated BMT. Therefore, we retrospectively compared the Cy-TBI and Bu-Cy regimens in a large series of patients who underwent unrelated BMT in Japan. We also evaluated the efficacy of total lymphoid irradiation (TLI), which was added to the Bu-Cy regimen to prevent engraftment failure. Another object of this study was to evaluate the effect of an intensified conditioning regimen in which antineoplastic agents other than Cy, such as cytarabine, etoposide, and Bu, were added to the Cy-TBI regimen [13-15].

PATIENTS AND METHODS

Study Population and Transplantation Procedure

A total of 3543 patients who underwent allogeneic BMT from an unrelated donor between 1993 and 2002 for CML, AML, acute lymphoblastic leukemia (ALL), or myelodysplastic syndrome (MDS) were reported to the Japan Marrow Donor Program [16-18]. Those <16 years of age, those who received a manipulated graft, and those who received antithymocyte globulin or alemtuzumab as a part of their conditioning regimen were excluded from the study. The conditioning regimens before transplantation were classified into the following groups. The Cy-TBI regimen was defined as the combination of Cy and TBI only. The total dose of Cy was between 100 and 150 mg/kg. The total dose of TBI was between 10 and 15 Gy. Cy-TBI$^+$ regimens were defined as those that included another antineoplastic agent added to the Cy-TBI regimen. The added agent was cytarabine in 61%, etoposide in 14%, and Bu in 24% of cases. The Bu-Cy regimen was defined as the combination of Bu and Cy. The total dose of Cy was between 100 and 150 mg/kg. The total dose of Bu was 16 mg/kg in most patients. The Bu-Cy-TLI regimen was the combination of Bu-Cy and TLI. TLI was typically performed at 5 to 8 Gy in 1 or 2 fractions. Patients who received a conditioning regimen that did not belong to these groups were excluded from the analysis. Finally, 1875 patients were included in the study.

The conditioning regimen was chosen at the discretion of each center. Bone marrow was exclusively used as a stem cell source. Prophylaxis for graft-versus-host disease (GVHD) mainly consisted of a combination of cyclosporin A and methotrexate (60%) or a combination of tacrolimus and methotrexate (32%).

Statistical Considerations

Data were collected by the Japan Marrow Donor Program by using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and annually after transplantation. Data for August 2003 were used in the following analyses. The primary end point was survival after transplantation. The incidences of engraftment failure and grade III/IV acute GVHD, which was graded according to the published criteria [19], were secondary end points. Engraftment was defined as a neutrophil count >500/μL for 3 consecutive days after transplantation. Engraftment failure was diagnosed when engraftment was not achieved at any time after transplantation. The incidences of secondary graft failure, defined as persistent neutropenia after engraftment and acute GVHD, were analyzed in 1744 patients who achieved initial engraftment.

The probability of survival and the cumulative incidence of acute GVHD were calculated with the Kaplan-Meier method. The cumulative incidence of relapse was calculated by Gray's method by considering death without relapse as a competing risk [20]. Univariate comparison for dichotomous variables between groups was performed with the Fisher exact test or the χ^2 test, and comparisons for time-to-event variables were performed with the log-rank test. Univariate analyses to compare the type of conditioning regimen were performed to test the null hypothesis that the effects of each conditioning regimen were the same. Multivariate analyses for dichotomous and time-to-event variables were performed by using logistic regression analysis and proportional hazards modeling, respectively. Potential confounding factors considered in the analysis included recipient age, re-

Table 1. *Characteristics of the Patients (N = 1875)*

Variable	Cy-TBI (n = 714)	Cy-TBI$^+$ (n = 861)	Bu-Cy (n = 243)	Bu-Cy-TLI (n = 57)	*P* Value
Recipient sex male	63%	62%	54%	54%	.069
Recipient age ≥40 y	35%	27%	37%	44%	.0005
Donor sex male	62%	63%	61%	56%	.67
Donor age ≥40 y	26%	28%	30%	25%	.56
HLA-antigen mismatch in HVG direction	4.6%	4.3%	4.8%	2.0%	.85
HLA-antigen mismatch in GVH direction	3.0%	3.7%	2.7%	2.0%	.82
HLA-allele mismatch in HVG direction	35%	37%	30%	24%	.13
HLA-allele mismatch in GVH direction	35%	37%	31%	29%	.44
ABO major mismatch	27%	28%	27%	27%	.98
ABO minor mismatch	23%	25%	21%	30%	.41
Diagnosis					
ALL	24%	33%	10%	0%	<.0001
AML	30%	31%	32%	37%	
CML	32%	26%	44%	53%	
MDS	15%	10%	14%	11%	
High-risk category	17%	34%	20%	16%	<.0001
CMV serostatus positive	80%	80%	81%	76%	.81
Cell dose in the graft ≥3.0 × 10^8 cells/kg	46%	53%	49%	46%	.047
GVHD prophylaxis: tacrolimus + methotrexate	35%	37%	32%	9%	.0004
G-CSF used	85%	86%	86%	51%	<.0001

cipient sex, donor age, donor sex, underlying disease, disease status, serologic/genotypic HLA mismatch, ABO mismatch, cytomegalovirus serostatus, conditioning regimen, cell dose in the graft, GVHD prophylaxis regimen, and the use of granulocyte colony-stimulating factor (G-CSF). Acute leukemia in first or second remission, CML in first or second chronic phase, and MDS without leukemic transformation were considered standard-risk diseases, whereas others were considered high-risk diseases [21]. An HLA mismatch in the graft-versus-host (GVH) direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas mismatch in the host-versus-graft (HVG) direction was defined as when the donor's antigens or alleles were not shared by the recipient. HLA-allele mismatch included the presence of HLA mismatch at both the antigen and allele levels. Factors other than the type of conditioning regimen that showed at least borderline significance ($P < .10$) in univariate analyses were included in the multivariate analyses and then deleted stepwise from the model, except that underlying disease was consistently kept in the model. The type of conditioning regimen was added in the final model.

RESULTS

Characteristics of the Patients

The median age of the 1875 eligible patients was 33 years (range, 16-63 years). The number of patients who received the Cy-TBI, Cy-TBI$^+$, Bu-Cy, and Bu-Cy-TLI regimens was 714, 861, 243, and 57, respectively (Table 1). A significant difference in the patients' background characteristics was observed with regard to recipient age, diagnosis, disease risk category, GVHD prophylaxis regimen, and the use of G-CSF after transplantation. The Cy-TBI$^+$ regimen tended to be used in younger patients and high-risk patients. The use of non-TBI regimens was less frequent in ALL. The lower incidence of FK506-based GVHD prophylaxis and posttransplant G-CSF in patients who received the Bu-Cy-TLI regimen was probably due to each center's policy.

Engraftment Failure

Engraftment failure was observed in 65 patients (3.6%). Univariate analysis identified 5 risk factors that affected the incidence of engraftment failure with a *P* value of <.10: higher recipient age, HLA-allele mismatch in the HVG direction, ABO major mismatch, high-risk disease, and low cell dose in the graft. By a multivariate analysis, all of these factors except for ABO major mismatch were identified as independent significant risk factors for engraftment failure (Table 2). When we added the type of conditioning regimen to this model, the use of a Bu-Cy regimen significantly increased the incidence of engraftment failure (odds ratio [OR], 2.49; 95% confidence interval [CI], 1.02-6.13; $P = .046$). There was no significant difference in the time to engraftment among the conditioning regimen groups ($P = .26$; Figure 1A).

Secondary graft failure was observed in 1.6% of patients who achieved initial engraftment. Logistic regression analysis revealed that only higher donor age was an independent significant risk factor for secondary graft failure (OR, 2.38; 95% CI, 1.07-5.29; $P = .034$). The type of conditioning did not significantly affect the incidence of secondary graft failure.

Table 2. *Multivariate Analyses for Engraftment Failure, Grade III/IV Acute GVHD, and Overall Survival before and after Adding the Type of Conditioning Regimen to the Model*

Factor	Before		After	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Engraftment failure				
Recipient age				
<40 y	1.00		1.00	
≥40 y	2.00 (1.05-3.80)	.035	2.02 (1.06-3.88)	.032
HLA-allele mismatch in HVG direction				
No	1.00		1.00	
Yes	3.36 (1.74-6.47)	.0003	3.33 (1.72-6.45)	.0003
Risk category				
Standard	1.00		1.00	
High	3.10 (1.50-6.41)	.0023	3.40 (1.61-7.14)	.0014
Cell dose				
$<3.0 \times 10^8$ cells/kg	1.00		1.00	
$\geq 3.0 \times 10^8$ cells/kg	0.36 (0.18-0.71)	.0036	0.36 (0.18-0.73)	.0046
Diagnosis				
ALL	1.00			
AML	0.42 (0.16-1.06)	.066	0.38 (0.15-0.98)	.045
CML	0.58 (0.24-1.36)	.21	0.50 (0.21-1.21)	.12
MDS	2.51 (0.95-6.62)	.063	2.33 (0.87-6.25)	.094
Regimen				
Cy-TBI			1.00	
Cy-TBI$^+$			0.87 (0.41-1.83)	.71
Bu-Cy			2.49 (1.02-6.13)	.046
Bu-Cy-TLI			0.00	.98
	RR (95% CI)	P Value	RR (95% CI)	P Value
Grade III/IV acute GVHD				
HLA allele mismatch in GVH direction				
No	1.00		1.00	
Yes	1.95 (1.47-2.57)	<.0001	1.96 (1.48-2.59)	<.0001
ABO-minor mismatch				
No	1.00		1.00	
Yes	1.36 (1.01-1.82)	.045	1.36 (1.01-1.83)	.043
GVHD prophylaxis				
Cyclosporine + methotrexate	1.00		1.00	
Tacrolimus + methotrexate	0.53 (0.38-0.74)	<.0002	0.53 (0.38-0.74)	.0002
Diagnosis				
ALL	1.00		1.00	
AML	0.86 (0.55-1.32)	.48	0.86 (0.55-1.33)	.49
CML	1.86 (1.29-2.67)	.0009	1.85 (1.27-2.68)	.0014
MDS	1.41 (0.84-2.38)	.19	1.44 (0.85-2.44)	.17
Regimen				
Cy-TBI			1.00	
Cy-TBI$^+$			1.19 (0.87-1.63)	.29
Bu-Cy			1.28 (0.83-1.98)	.27
Bu-Cy-TLI			1.18 (0.58-2.38)	.65
	RR (95% CI)	P Value	RR (95% CI)	P Value
Overall survival				
Recipient age				
<40 y	1.00		1.00	
≥40 y	1.50 (1.27-1.77)	<.0001	1.54 (1.31-1.83)	<.0001
Donor age				
<40 y	1.00		1.00	
≥40 y	1.20 (1.01-1.42)	.036	1.17 (0.99-1.38)	.074
HLA-allele mismatch in GVH direction				
No	1.00		1.00	
Yes	1.55 (1.32-1.82)	<.0001	1.56 (1.33-1.83)	<.0001
G-CSF				
No	1.00			
Yes	1.30 (1.04-1.64)	.024	1.32 (1.05-1.67)	.020
Risk category				
Standard	1.00		1.00	
High	2.48 (2.09-2.93)	<.0001	2.31 (1.95-2.76)	<.0001
GVHD prophylaxis				
Cyclosporine + methotrexate	1.00		1.00	
Tacrolimus + methotrexate	0.73 (0.62-0.87)	.0005	0.72 (0.60-0.86)	.0003

Table 2. *(Cont'd)*

Factor	Before		After	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Diagnosis				
ALL	1.00		1.00	
AML	0.86 (0.69-1.07)	.18	0.88 (0.70-1.09)	.24
CML	0.82 (0.66-1.01)	.063	0.84 (0.68-1.04)	.11
MDS	1.24 (0.93-1.66)	.13	1.30 (0.98-1.74)	.070
Regimen				
Cy-TBI			1.00	
Cy-TBI$^+$			1.45 (1.20-1.74)	<.0001
Bu-Cy			1.31 (1.00-1.73)	.050
Bu-Cy-TLI			1.43 (0.91-2.26)	.12

Acute and Chronic GVHD

The incidence of grade II to IV and grade III/IV acute GVHD was 43.9% and 16.7%, respectively. Male sex, higher donor age, HLA mismatch in the GVH direction, ABO minor mismatch, underlying disease, high-risk disease, and the GVHD prophylaxis regimen affected the incidence of grade III/IV acute GVHD with at least borderline significance ($P < .10$). Among these, HLA-allele mismatch in the GVH direction, ABO minor mismatch, underlying disease, and GVHD prophylaxis were identified as independent risk factors by a multivariate analysis (Table 2). There was no difference in the incidence of acute GVHD among the 4 types of conditioning regimens after adjustment for these risk factors (Table 2 and Figure 1B).

Chronic GVHD was observed in 49.7% of patients who achieved engraftment and survived disease free for at least 100 days after transplantation. Only the presence of an HLA-allele mismatch in the GVH direction significantly affected the incidence of chronic GVHD by multivariate analysis. The type of conditioning did not significantly affect the incidence of chronic GVHD.

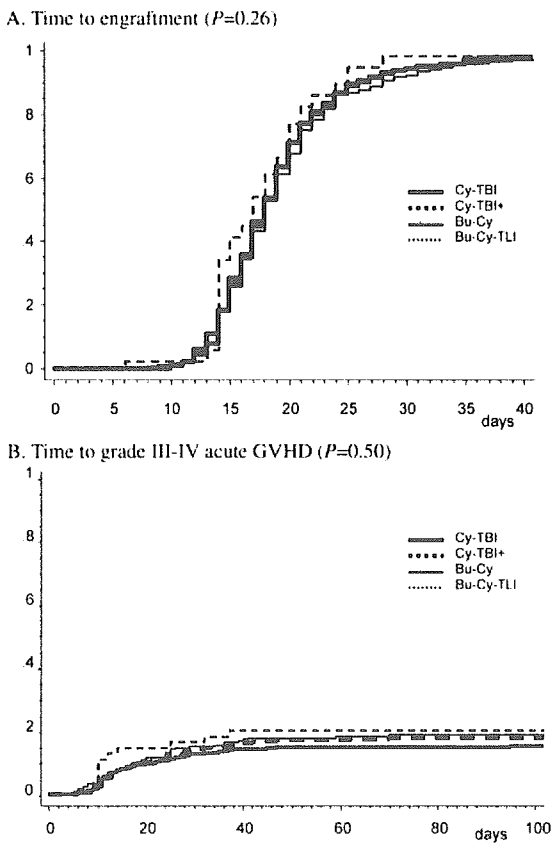

Figure 1. Days to engraftment (A) and days to grade III/IV acute GVHD (B) grouped according to the type of conditioning regimen.

Survival after Transplantation

Overall survival and disease-free survival at 5 years after transplantation for all of the patients was 46.2% and 42.5%, respectively. Overall survival stratified by disease status, grouped according to the conditioning regimen, is shown in Figure 2. A significant difference in survival was observed in standard-risk patients. Risk factors for shorter survival with a P value of <.10 identified by the log-rank test included male sex, higher recipient age, higher donor age, HLA mismatch in both the GVH and HVG directions, ABO major mismatch, high-risk disease, cytomegalovirus seropositivity, use of G-CSF after transplantation, and GVHD prophylaxis consisting of cyclosporin A and methotrexate. Proportional hazard modeling identified 6 independent significant risk factors: higher patient age, higher donor age, HLA-allele mismatch in the GVH direction, use of G-CSF, high-risk disease, and the use of the combination of cyclosporin A and methotrexate (Table 2). When we added the type of conditioning regimen to the proportional hazard model, the Cy-TBI$^+$ and Bu-Cy regimens were significantly inferior to the Cy-TBI regimen (relative risk [RR], 1.45; 95% CI, 1.20-1.74; $P < .0001$ and RR, 1.31; 95% CI, 1.00-1.73; $P = .050$, respectively).

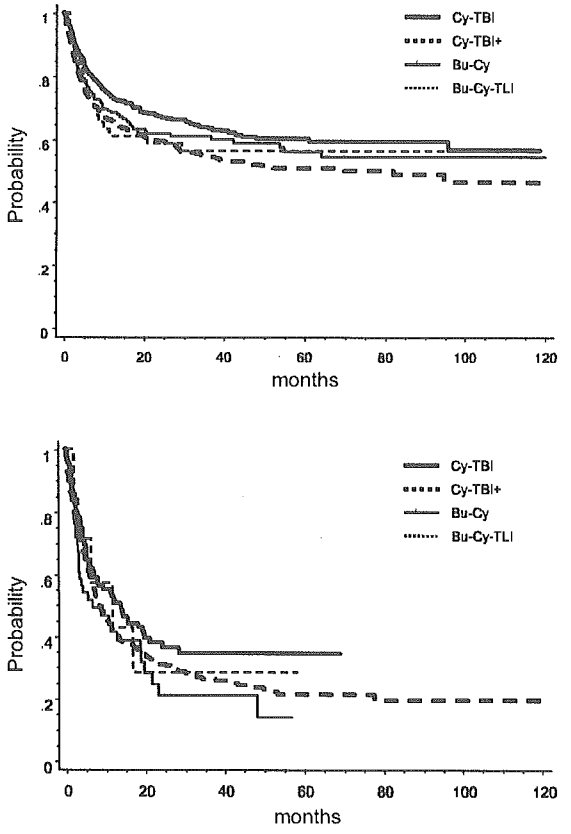

Figure 2. Overall survival grouped according to the type of conditioning regimen in standard-risk (A) and high-risk (B) patients.

Analyses Based on Detailed HLA Matching

We added analyses based on detailed HLA matching because it has been reported that the outcome of unrelated BMT is affected not only by the presence of HLA-allele mismatch, but also by whether the HLA-allele mismatch belongs to class I or class II [16,18]. In this study, none of the HLA-A/-B antigen, HLA-C antigen, HLA-DR antigen, HLA-A/-B allele, HLA-C allele, or HLA-DRB1 allele mismatches in the HVG direction significantly affected the incidence of engraftment failure, probably because of the small number of patients in each group. However, mismatches in the GVH direction at the HLA-A/-B antigen, HLA-C antigen, HLA-A/-B allele, HLA-C allele, and HLA-DRB1 allele significantly affected the incidence of grade III/IV acute GVHD in univariate analyses. These factors were included in the multivariate analysis, and HLA-A/-B allele, HLA-C allele, and HLA-DRB1 allele mismatches were shown to be independently significant. However, the effect of the conditioning regimen on the incidence of grade III/IV acute GVHD was not significant after adjustment for the independent significant factors. As for survival after transplantation, mismatches in both the HVG and GVH directions at the HLA-A/-B antigen, HLA-C antigen, HLA-A/-B allele, HLA-C allele, and HLA-DRB1 allele significantly affected overall survival in univariate analyses. Among these, the presence of an HLA-A/-B antigen mismatch in the HVG direction and an HLA-A/-B allele mismatch in the GVH direction were identified as independent significant risk factors for overall survival. After adjustment for these factors, as well as other independent significant risk factors, the adverse effects of the Cy-TBI$^+$ and Bu-Cy regimens remained significant (RR, 1.42; 95% CI, 1.18-1.70; $P = .0002$ and RR, 1.31; 95% CI, 1.00-1.72; $P = .052$, respectively).

Other Statistical Analyses to Ensure the Results

We added statistical analyses to ensure the findings of this study. First, we repeated the analyses by using only patients who received the Cy-TBI or Bu-Cy regimen, to confirm the difference between the 2 regimens. The findings were almost the same, and the use of Bu-Cy adversely affected the incidence of engraftment failure and overall survival (OR, 2.53; 95% CI, 1.00-6.39; $P = .049$ and RR, 1.32; 95% CI, 1.00-1.75; $P = .053$, respectively).

Next, we changed the method of the multivariate analyses to include all factors with at least borderline significance ($P < .10$) in univariate analyses, as well as the underlying disease and the type of conditioning regimen, followed by a stepwise deletion of nonsignificant factors. This change in the statistical method did not change the major findings of this study. The Bu-Cy regimen was inferior to the Cy-TBI regimen in the incidence of engraftment failure and overall survival (OR, 2.49; 95% CI, 1.02-6.12; $P = .045$ and RR, 1.33; 95% CI, 1.02-1.75; $P = .046$, respectively). The Cy-TBI$^+$ regimen was inferior to the Cy-TBI regimen in overall survival (RR, 1.46; 95% CI, 1.21-1.75; $P < .0001$).

Relapse and Nonrelapse Mortality

To evaluate the cause of the difference in survival among the different types of conditioning regimens, we further analyzed the incidences of relapse and nonrelapse mortality. Multivariate analyses revealed that the incidence of relapse after the Bu-Cy-TLI regimen was significantly lower than that after the Cy-TBI regimen (RR, 0.13; 95% CI, 0.02-0.90; $P = .039$, adjusted for ABO major mismatch, underlying disease, disease status, and GVHD prophylaxis), although this benefit was offset by a significant increase in the incidence of nonrelapse mortality (RR, 1.89; 95% CI, 1.20-3.00; $P = .0061$, adjusted for recipient age, donor age, underlying disease, disease status, HLA-allele mismatch in the HVG direction, G-CSF, and GVHD prophylaxis); this resulted in similar survival. The incidence of nonrelapse mortality after the Cy-TBI$^+$ regimen was significantly higher than that after the Cy-TBI regimen (RR, 1.48; 95% CI, 1.20-

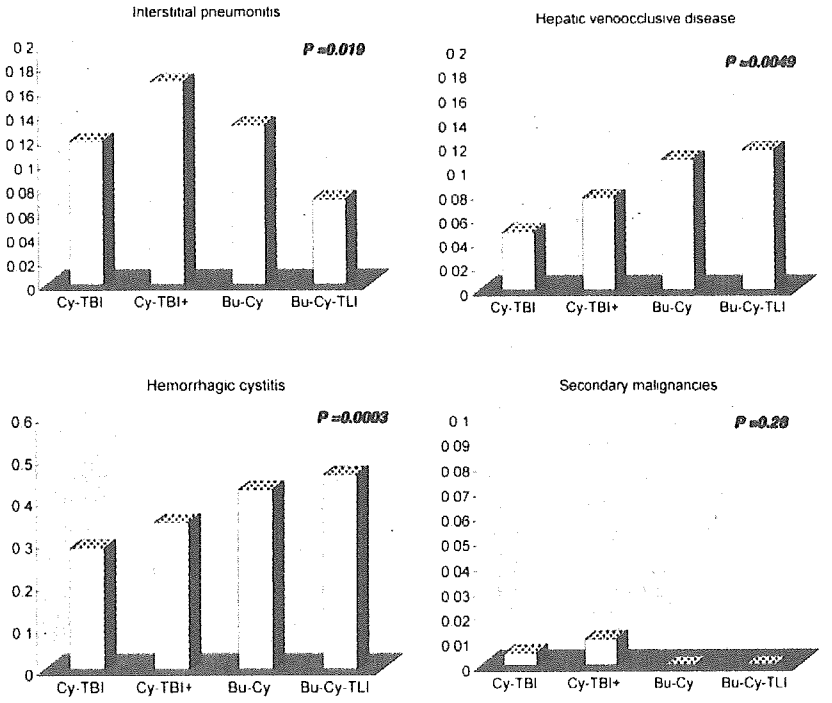

Figure 3. Incidence of interstitial pneumonitis, hepatic veno-occlusive disease, and secondary malignancies, excluding posttransplantation lymphoproliferative disorders.

1.84; P = .0003, adjusted as described previously), whereas there was no difference in the incidence of relapse (RR, 0.84; 95% CI, 0.64-1.11; P = .22). There was no significant difference in the incidence of relapse and nonrelapse mortality between the Cy-TBI and Bu-Cy regimens (RR, 0.89; 95% CI, 0.57-1.38; P = .59 and RR, 1.21; 95% CI, 0.89-1.65; P = .23, respectively).

Other Complications after Transplantation

The incidence of interstitial pneumonitis was significantly different among the 4 conditioning regimens (P = .019; Figure 3). The incidence of interstitial pneumonitis after the Cy-TBI$^+$ regimen was significantly higher than that after the Cy-TBI regimen (OR, 1.59; 95% CI, 1.13-2.23; P = .0076, adjusted for underlying disease, HLA-allele mismatch in the HVG direction, and GVHD prophylaxis). A statistically significant difference was not observed between the Cy-TBI and Bu-Cy regimens (P = .66). The incidence of VOD was also significantly different among the 4 conditioning groups (P = .0049). It was significantly higher after the Cy-TBI$^+$, Bu-Cy, and Bu-Cy-TLI regimens than after the Cy-TBI regimen (OR, 1.64; 95% CI, 1.00-2.71; P = .052; OR, 3.00; 95% CI, 1.62-5.45; P = .0005; and OR, 3.20; 95% CI, 1.11-8.24; P = .032, respectively, adjusted for underlying disease, HLA-allele mismatch in the HVG direction, ABO major mismatch, ABO minor mismatch, and G-CSF). The incidence of hemorrhagic cystitis was significantly affected by the type of conditioning regimen (P = .0003). It was also significantly higher after the Cy-TBI$^+$, Bu-Cy, and Bu-Cy-TLI regimens than after the Cy-TBI regimen (OR, 1.37; 95% CI, 1.09-1.72; P = .0075; OR, 1.85; 95% CI, 1.34-2.56; P = .0002; and OR, 2.11; 95% CI, 1.16-3.85; P = .015, respectively, adjusted for underlying disease and donor sex).

Secondary malignancies excluding posttransplantation lymphoproliferative disorders developed in 8 patients a median of 35 months (range, 15-84 months) after transplantation, including MDS in 2 and AML, thyroid cancer, uterine body cancer, esophageal cancer, breast cancer, and squamous cell cancer in 1 each. The incidence of secondary malignancies was not significantly different among the 4 conditioning groups.

DISCUSSION

In this study, we retrospectively evaluated the effect of the conditioning regimen on the outcome of unrelated BMT. The Cy-TBI regimen was superior to the Bu-Cy regimen, not only with regard to the incidence of engraftment failure, but also for overall survival after transplantation. The addition of TLI to the Bu-Cy regimen decreased the incidences of engraftment failure and relapse but increased nonrelapse mortality. Intensified conditioning regimens in which

another antineoplastic agent was added to the Cy-TBI regimen resulted in increased nonrelapse mortality and inferior survival.

On the basis of the results of randomized controlled trials and their meta-analysis, the Cy-TBI regimen is generally preferred to the Bu-Cy regimen except for patients with CML in chronic phase in HSCT from an HLA-identical sibling donor [5-10]. This study showed that Cy-TBI may be the first-choice regimen in most patients who undergo unrelated BMT unless the patient has a condition that precludes the use of TBI, such as previous high-dose irradiation to a major organ. The weakness of the Bu-Cy regimen was apparent in the increased incidences of engraftment failure and VOD. As a current general practice in Japan, Bu is administered orally without monitoring the plasma concentration. Therefore, the use of intravenous Bu or oral Bu targeted to a predetermined plasma level may improve the outcome after the Bu-Cy regimen [22]. However, further trials are required to evaluate the efficacy of intravenous Bu and targeted oral Bu.

Higher nonrelapse mortality after the intensified Cy-TBI^{+} regimen might reflect the possibility that the regimen was preferentially used in patients with advanced diseases. However, the incidence of nonrelapse mortality was significantly higher after adjustment for disease status and also when the comparison was limited to patients with standard-risk disease (RR, 1.47; 95% CI, 1.14-1.90; $P = .0031$). Conversely, a decrease in the relapse incidence was not observed either in standard-risk or in high-risk patients (RR, 0.81; 95% CI, 0.57-1.15; $P = .24$ and RR, 0.89; 95% CI, 0.57-1.39; $P = .60$). Therefore, these results did not show any benefit for the intensified regimens.

This was a retrospective study, and it was impossible to completely eradicate biases. First, non-TBI regimens were preferentially used in older patients. Second, the use of Bu-based regimens was less frequent in ALL compared with myeloid malignancies. Third, the intensified Cy-TBI^{+} regimen was most frequently used in young patients with high-risk diseases. Therefore, we adjusted the effect of the conditioning regimen for these variables in multivariate analyses. We should also consider the "center" effect as a possible bias. However, a study from the Japan Society for HSCT did not show a significant center effect in unrelated BMT in Japan [23]. The inclusion of patients who underwent transplantation from 1993 and 2002 might have resulted in the significant variations in transplantation procedures. We could not obtain detailed information of supportive care, and this is one of the limitations of this type of registry data study.

The use of G-CSF after transplantation significantly adversely affected survival. A similar result was observed in a retrospective study by the European Group for Blood and Marrow Transplantation [24]. However, such an adverse effect has not been shown in prospective randomized controlled trials that evaluated the use of G-CSF after transplantation [25]. Patients with preexisting infections or other comorbidities might have tended to receive G-CSF. These data were not included in the analyses and thus might have biased the results.

Although a definite conclusion cannot be made without a randomized controlled trial, >1000 patients will be required to detect the meaningful difference (RR, 1.31) in survival between the Cy-TBI and Bu-Cy groups that was seen in this study at a statistically significant level with α and β errors of 5% and 20%, respectively. Thus, realistically, this retrospective study that considered possible biases in multivariate analyses may be the best evidence. More than 30 years have passed since the introduction of the Cy-TBI regimen. Nevertheless, the Cy-TBI regimen still seems to be the most suitable regimen not only in HSCT from an HLA-identical sibling donor, but also in unrelated BMT.

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High-grade cytomegalovirus antigenemia after hematopoietic stem cell transplantation

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Summary:

Clinical impact of high-grade (HG) cytomegalovirus (CMV) antigenemia after hematopoietic stem cell transplantation has not been clarified. Therefore, in order to investigate the risk factors and outcome for HG-CMV antigenemia, we retrospectively analyzed the records of 154 Japanese adult patients who underwent allogeneic hematopoietic stem cell transplantation for the first time from 1995 to 2002 at the University of Tokyo Hospital. Among 107 patients who developed positive CMV antigenemia at any level, 74 received risk-adapted preemptive therapy with ganciclovir (GCV), and 17 of these developed HG-antigenemia defined as ⩾50 positive cells per two slides. The use of systemic corticosteroids at ⩾0.5 mg/kg/day at the initiation of GCV was identified as an independent significant risk factor for HG-antigenemia. Seven of the 17 HG-antigenemia patients developed CMV disease, with a cumulative incidence of 49.5%, which was significantly higher than that in the low-grade antigenemia patients (4%, $P<0.001$). However, overall survival was almost equivalent in the two groups. In conclusion, the development of HG-antigenemia appeared to depend on the profound immune suppression of the recipient. Although CMV disease frequently developed in HG-antigenemia patients, antiviral therapy could prevent a fatal outcome.

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Cytomegalovirus (CMV) disease is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Two strategies have been tested for the prevention of CMV disease after HSCT. First, universal prophylaxis with ganciclovir (GCV) after engraftment has led to a decrease in early CMV disease.[1,2] However, it also increased invasive fungal infections due to neutropenia and did not improve survival. Alternatively, preemptive therapy with GCV only in patients who are at high risk for CMV disease was investigated. Preemptive therapy with monitoring of the CMV viral load by antigenemia assay or polymerase chain reaction (PCR) resulted in a marked reduction of CMV disease without a significant increase in the incidence of bacterial or fungal infections.[3,4]

Although the level of CMV was expected to predict the development of CMV disease,[5] there has been some discrepancy regarding the correlation between the level of CMV antigenemia and the clinical outcome. Nichols *et al*[6] reported that rising levels of CMV antigenemia during preemptive therapy did not correlate with CMV disease among allogeneic HSCT recipients. On the other hand, other studies have shown a significant correlation between high viral load and CMV disease.[7,8] Therefore, we retrospectively analyzed the clinical impact of high-grade (HG) CMV antigenemia in allogeneic HSCT patients.

Patients and methods

Study population

We analyzed the records of 154 consecutive adult patients (⩾16 years old) who underwent allogeneic HSCT from an HLA-matched or a one-locus-mismatched donor for the first time at the University of Tokyo Hospital between June 1995 and December 2002. Nine patients who received reduced-intensity conditioning were included. The patient characteristics are shown in Table 1. In total, 66, 23, and 65 patients received graft from an HLA-matched related donor, a one-locus-mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 33 related donors chose to donate peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered as high-risk diseases. Donors other than HLA-matched related donors were defined as alternative donors.

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Table 1 Patients' characteristics

Characteristic	*Total patients*
Sex (male/female)	106/48
Age, median (range)	35.0 (16–60)
Serostatus before transplant	
Recipient CMV-positive/negative	137/17
Donor CMV-positive/negative	129/25
Underlying disease	
Acute leukemia	73
CML	40
MDS	17
NHL/ATL	11
SAA	8
Other	5
Graft source	
PBSC	33
BM	121
Donor type	
Matched related	66
Mismatched related	23
Unrelated	65
Preparative regimen	
CY/TBI – based regimen	104
BU/CY – based regimen	34
ATG – including regimen	7
Nonmyeloablative regimen	9
GVHD prophylaxis	
CyA + MTX	137
Tacrolimus + MTX	17
Acute GVHD	
Grades 0–I	85
Grades II–IV	69

CMV = cytomegalovirus; PBSC = peripheral blood stem cell; BM = bone marrow; CY = cyclophosphamide; TBI = total body irradiation; BU = busulfan; ATG = antithymocyte globulin; GVHD = graft-versus-host disease; CyA = cyclosporine; MTX = methotrexate.

Transplantation procedure

The preparative regimen for leukemia/lymphoma was mainly performed with either a total body irradiation (TBI) regimen (cyclophosphamide (Cy) at 60 mg/kg for 2 days and TBI at 200 cGy twice daily for 3 days) or a non-TBI regimen (Cy at the same dose combined with busulfan (Bu) at 4 mg/kg for 4 days). Reduced-intensity regimens included the FB regimen (fludarabine (Flu) at 30 mg/m^2 for 6 days and Bu at 4 mg/kg for 2 days) and the FB16 regimen (Flu at the same dose with Bu 4 mg/kg for 4 days), and were used for elderly or clinically infirm patients.[9] Gemcitabine at 1000 mg/m^2 for 3 days was added to the FB regimen for patients with pancreatic cancer.[10] The anti-thymocyte globulin (ATG) regimen for aplastic anemia consisted of Cy at 50 mg/kg for 4 days and rabbit ATG at 5 mg/kg for 5 days with or without TBI at 200 cGy.

For prophylaxis against graft-versus-host disease (GVHD), cyclosporine A at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was administered combined with short-term methotrexate (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and optionally on day 11). Methylprednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD. Prophylaxis against bacterial, fungal, herpes virus, and *Pneumocystis carinii* infections consisted of fluconazole, tosufloxacin, acyclovir, and sulfamethoxazole/trimethoprim.

CMV antigenemia assay

CMV antigenemia assay was performed at least once a week after engraftment as described previously.[11] In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with monoclonal antibody C10/11 (Clonab CMV; Biotest, Dreieich, Germany), which targets CMV pp65 antigen, and reacted with goat alkaline phosphatase-labeled anti-mouse immunoglobulin (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under light microscopy, CMV-positive cells were counted and the results are presented as the number of positive cells per two slides.

Preemptive therapy for CMV disease

Preemptive therapy against CMV disease was performed by monitoring CMV antigenemia weekly after engraftment. Until June 2001, intravenous GCV was started at an induction dose of 10 mg/kg/day when 10 or more CMV-positive cells were detected in patients who underwent HSCT from an HLA-matched related donor and when positive cells were detected at any level in patients who underwent HSCT from an alternative donor. From July 2001, the induction dose was decreased to 5 mg/kg/day and the threshold of antigenemia to start GCV was changed to 20 and three positive cells for patients who underwent HSCT from an HLA-matched related donor and an alternative donor, respectively.[12] The dose of GCV was increased to 10 mg/kg/day when rising antigenemia was observed. The dose of GCV was adjusted according to the weekly monitoring of the creatinine clearance. GCV was continued until negative antigenemia was observed. Foscarnet at an induction dose of 120 mg/kg/day was substituted for GCV for patients with severe neutropenia or progressive CMV infection during GCV administration.

Definition of HG-CMV antigenemia and CMV disease

Positive antigenemia was defined as a detection of CMV-positive cells at any level. HG-antigenemia was defined as a positive result with 50 or more positive cells per two slides and low-grade (LG) antigenemia as the presence of less than 50 positive cells, because the 75 percentile value of maximal antigenemia in each patient was 48 positive cells per two slides. All patients with symptoms compatible with CMV disease such as interstitial pneumonia, colitis, and gastritis underwent extensive pathological examination of biopsy specimens. Patients with symptoms compatible with CMV retinitis received opthalmoscopy and/or PCR to detect CMV-DNA using aqueous humor, to establish a definite diagnosis of CMV disease.

Statistical analysis

Univariate and multivariate analyses for time-to-event covariates were performed using the log-rank test and proportional-hazard modeling, respectively. Factors associated with at least borderline significance ($P<0.10$) in univariate analyses were subjected to a multivariate analysis and stepwisely deleted from the model. The cumulative incidence of CMV disease was evaluated using Gray's method, considering death without CMV disease as a competing risk.[13]

Results

Incidence and risk factors for positive CMV antigenemia

Of the 154 patients, 107 (69.5%) developed positive antigenemia at a median of 42 days (range 12–637 days) after transplantation. In univariate analyses, higher age, recipient CMV seropositivity, HLA disparity, HSCT from an alternative donor, and grades II–IV acute GVHD were associated with the development of positive antigenemia (Table 2). In a multivariate analysis, recipient CMV seropositivity, HLA disparity, and grades II–IV acute GVHD were identified as independent risk factors for positive antigenemia.

Incidence and risk factors for HG-CMV antigenemia

In total, 74 patients received GCV as preemptive therapy, and 17 of these developed HG-antigenemia at a median of 49 days (range 36–637 days) after HSCT. The use of tacrolimus, grades II–IV acute GVHD, and the use of systemic corticosteroids at any doses, $\geqslant 0.5$ mg/kg/day and $\geqslant 1.0$ mg/kg/day, upon the initiation of GCV were associated with a high incidence of HG-antigenemia with at

Table 2 Risk factors for positive CMV antigenemia

Univariate analysis *Factors*	*Variables*	*n*	*Incidence (%)*	*P-value*
Age	<40 years old	90	68	0.05
	⩾40 years old	64	83	
Donor CMV serostatus	Donor (−)	25	81	0.27
	Donor (+)	129	73	
Recipient CMV serostatus	Recipient (−)	17	33	0.001
	Recipient (+)	137	79	
Donor type	Identical sibling donor	66	69	0.05
	Alternative donor	88	79	
HLA	Match	103	70	0.04
	Mismatch	51	85	
Disease risk	High	81	80	0.69
	Low	73	70	
Graft source	Bone marrow	121	70	0.13
	Peripheral blood	33	100	
Preparative regimen (1)	Non-ATG containing	147	74	0.76
	ATG containing	7	82	
Preparative regimen (2)	Non-TBI regimen	116	66	0.18
	TBI regimen	38	78	
Acute GVHD	Grades 0–I	86	66	0.001
	Grades II–IV	68	85	
GVHD prophylaxis	CyA + MTX	137	74	0.56
	Tacrolimus + MTX	17	76	
Multivariate analysis *Factors*	*Relative risk*	*95% CI*	*P-value*	
Recipient CMV serostatus	5.2 (Positive *vs* negative)	2.1–13.0	<0.001	
Donor	1.5 (Alternative *vs* identical sib.)	1.0–2.3	0.03	
Grade II–IV acute GVHD	2.1 (Grades II–IV *vs* 0–I)	1.4–3.1	<0.001	

least borderline significance (Table 3). Among these, the use of systemic corticosteroids of ⩾0.5 mg/kg/day upon the initiation of GCV was the only independent risk factor for HG-antigenemia.

Clinical outcome of patients who developed HG-antigenemia

The median peak antigenemia level of the 17 patients who developed HG-antigenemia was 95 positive cells per two slides (range 50–821) (Table 4). They received preemptive GCV for a median duration of 22 days (range 9–72), all of whom developed HG-antigenemia after starting GCV. A total of 16 patients had been receiving corticosteroids at the detection of HG-antigenemia, whereas the remaining one who had received ATG as conditioning had not been taking corticosteroid. GCV was replaced with foscarnet in four patients with persistent HG-antigenemia or severe neutropenia during GCV administration. Five patients developed recurrent HG-antigenemia at a median of 70.5 days after the first episode.

Seven of the 17 HG-antigenemia patients developed CMV disease with a cumulative incidence of 49.5%, at a median onset of 50 days (range 0–309 days) from the initiation of GCV. CMV pneumonia developed in three patients, colitis in four, gastritis in one, and retinitis in one (Table 4). The incidence of CMV disease was significantly higher in patients who developed HG-antigenemia than in

Table 3 Risk factors for high-grade CMV antigenemia among 74 patients who received preemptive ganciclovir after transplantation

Univariate analysis *Factors*	*Variables*	*n*	*Incidence (%)*	*P-value*
Age	<40 years old	39	26	0.68
	⩾40 years old	35	20	
Donor CMV serostatus	Donor (−)	15	15	0.33
	Donor (+)	59	23	
Recipient CMV serostatus	Recipient (−)	4	25	0.98
	Recipient (+)	70	23	
Donor type	Identical sibling donor	22	14	0.22
	Alternative donor	52	27	
HLA	Match	42	19	0.31
	Mismatch	32	29	
Disease risk	High	46	29	0.13
	Low	28	14	
Graft source	Bone marrow	57	20	0.11
	Peripheral blood	17	35	
Preparative regimen (1)	Non-ATG containing	72	23	0.35
	ATG containing	2	50	
Preparative regimen (2)	Non-TBI regimen	62	25	0.53
	TBI regimen	12	17	
Acute GVHD	Grades 0–I	29	10	0.04
	Grades II–IV	45	32	
GVHD prophylaxis	CyA + MTX	63	19	0.08
	Tacrolimus + MTX	11	47	
Steroid at any doses	None	22	5	0.04
	Done	52	31	
Steroid at ⩾0.5 mg/kg	None	35	9	0.007
	Done	39	36	
Steroid at ⩾1 mg/kg	None	45	16	0.09
	Done	29	35	
Initial dose of ganciclovir	10 mg/kg	45	18	0.15
	5 mg/kg	29	32	

Multivariate analysis *Factors*	*Relative risk*	*95% CI*	*P-value*
Steroids Use at ⩾0.5 mg/kg	4.60 (done *vs* none)	1.3–16.0	0.017