350

tool. With TDM, some of the unknown interindividual variations can be quantified and total fluctuations in drug exposure can be reduced after dose adjustment. There are several reports about TDM techniques of oral busulfan.[20,23,26]

To compare our data with the oral busulfan studies in adult patients, we used previously published data.[23] The selected study has similar sampling points to our study and individual AUC were reported. Several reports indicate that an AUC of 900–1500 μmol min/l[4,6,24,25] in patients receiving a conventional busulfan regimen prevents treatment failure and the risk of fatal toxicities. In the previous study, the observed AUC at a steady state in 8/12 (66.7%) patients without dose adjustment fell in the above range. The percentage of patients within the range increased to 92.9% (13/14) with the dose adjustment according to the TDM results. In our study, the observed AUCs at steady state in 25/29 (86.2%) patients were within the range.

TDM requires blood sampling, drug concentration measurement and pharmacokinetic analysis. In the case of TDM after the administration of busulfan, it takes 1 or 2 days to adjust the dosage regimen using TDM data. Therefore, 25–50% of busulfan exposure remains uncontrollable in the standard regimen of 16 doses four times per day for 4 days. The contribution of the TDM for busulfan therapy is limited because of the reason described above. Intravenous busulfan may have an advantage over the oral busulfan in the limited term therapy since the systemic exposure of intravenous busulfan is expected to be reproducible throughout the treatment as compared with that of oral busulfan with TDM.

Oral busulfan is generally used at a dose of 1 mg/kg in adults, but the recommended dosage of intravenous busulfan is 0.8 mg/kg.[10–12] The previous report suggests that 1 mg/kg oral busulfan is a slight over-dosage.[27] According to the previous report, the benefit of the intravenous busulfan administration is not only the decrease in the variability of systemic exposure (AUC), but also the optimizing of average exposure during busulfan dosing.[28] In order to come to a definite conclusion of the clinical benefit of the intravenous busulfan, a prospective comparison of exposure of intravenous versus oral administration might be needed.

Acknowledgements

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Graft-versus-Tumor Effect Against Advanced Pancreatic Cancer after Allogeneic Reduced-Intensity Stem Cell Transplantation

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Background. The prognosis of advanced pancreatic cancer is extremely poor and therefore a novel treatment strategy is desired. The authors thus started a prospective study of allogeneic reduced-intensity hematopoietic stem cell transplantation (RIST) for patients with advanced pancreatic cancer to evaluate the feasibility and efficacy of this approach for such patients.
Methods. Only patients with pathologically proven pancreatic cancer that was locally advanced or metastatic and not amenable to curative resection were included. The conditioning regimen consisted of gemcitabine, fludarabine, and busulfan.
Results. In the first stage of this study, the authors treated seven patients. Treatment-related mortality before day 100 was observed in one patient. The median survival after RIST was 229 days. An objective response on computed tomographic scan was observed in two patients and another had a tumor marker response. Marked tumor shrinkage was observed in one of the remaining patients after donor lymphocyte infusion. These antitumor effects appeared after the effect of the conditioning regimen had disappeared. In addition, some of these responses were associated with an increase in the serum anticarcinoembryonic antigen antibody level.
Conclusions. Pancreatic cancer appeared to be sensitive to a graft-versus-tumor effect; therefore, a larger clinical study with a refined strategy is warranted.

Keywords: Reduced-intensity stem cell transplantation, Minitransplantation, Pancreatic cancer, Graft-versus-tumor effect, Graft-versus-host disease.

Pancreatic cancer is the fifth most common cause of cancer-related mortality in Japan and the United States. The median duration of survival in advanced pancreatic cancer is less than 6 months, even when treated with gemcitabine (*1*), and therefore a novel treatment strategy is desired. Allogeneic nonmyeloablative or reduced-intensity hematopoietic stem cell transplantation (RIST) is a recently developed treatment approach for obtaining a graft-versus-tumor (GVT) effect without the toxicity associated with a myeloablative conditioning regimen (*2–6*). This treatment strategy is suitable for patients with solid tumors, because patients with advanced solid tumors are generally clinically infirm, and also a strong antitumor effect cannot be expected with an intensification of chemotherapy. In addition, a GVT effect has been noted in several solid tumors after conventional hematopoietic stem-cell transplantation (*7, 8*). Based on this background, RIST has been investigated for use against solid cancers since the late 1990s and its feasibility has already been demonstrated in

several studies (*9–14*). However, there is still little information available regarding its efficacy against individual solid cancers. Childs et al. showed an excellent response rate of 53% after RIST against metastatic renal cell cancer (*9*). A GVT effect against renal cell cancer was confirmed in trials by other centers. In contrast, RIST against metastatic melanoma, which has been considered to be a good candidate for immunotherapy, resulted in a miserable outcome (*15*). Therefore, it is difficult to predict whether a GVT effect can be achieved against an individual tumor. We started a prospective study to evaluate the feasibility and efficacy of RIST against advanced pancreatic cancer after ethical approval in April 2002.

PATIENTS AND METHODS

Patients

Eligible patients were younger than 70 years of age and had pathologically proven pancreatic adenocarcinoma, which was locally advanced or metastatic and not amenable to curative resection. Patients had a human leukocyte antigen (HLA)-matched sibling or a family donor with a single mismatched HLA antigen. Patients with a poor performance status (Eastern Cooperative Oncology Group 2–4) and those with severely impaired organ function were excluded. Patients and donors gave their written informed consent to participate in this study.

Twelve patients with pancreatic cancer fulfilled the inclusion criteria but lacked a suitable donor. They were considered control patients. Four of them had metastatic lesions, whereas eight had locally advanced disease. Nine received

chemotherapy with gemcitabine, whereas three were observed without chemotherapy.

Transplantation Procedure

Donors received granulocyte colony-stimulating factor at 200 $\mu g/m^2$ administered 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 2.0×10^6 CD34$^+$ cells/kg of the recipient's body weight were collected. Collected cells were then cryopreserved using standard techniques without ex vivo manipulation.

The conditioning regimen consisted of gemcitabine (1,000 mg/m^2/day as a 90-min infusion on days -16, -9, and -2) (16), fludarabine (30 mg/m^2/day as a 30-min infusion on days -8 to -3), and busulfan (4 mg/kg/day administered orally in four divided doses on days -6 and -5) (4, 17). Graft-versus-host disease (GVHD) prophylaxis was performed with cyclosporine (CsA) (3 mg/kg/day as a continuous infusion) and short-term methotrexate (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6). Frozen peripheral blood stem cells were thawed and infused on day 0. CsA was decreased at 10% per week from day 30 and discontinued by day 100 unless the patient developed acute GVHD. In patients with progressive disease without any evidence of acute GVHD, CsA was rapidly tapered over a 4-week period. Acute GVHD was graded as previously described (18). Grade II to IV acute GVHD was treated with methylprednisolone at 1 mg/kg per day, except for grade II GVHD limited to the skin, which was treated with topical steroid ointment.

Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of tosufloxacin, fluconazole, and sulfamethoxazole-trimethoprim. As prophylaxis against herpes simplex virus infection, acyclovir was administered at 500 mg per day intravenously or 1,000 mg per day orally from days -7 to 35, followed by long-term low-dose (200 mg/day) oral administration (19). Patients received granulocyte colony-stimulating factor (filgrastim) at 300 μg per day by 3-hr infusion beginning on day 7 until the neutrophil count recovered to 500/mm^3. Cytomegalovirus 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 (20, 21).

Donor lymphocyte infusion (DLI) was performed for patients who had progressive disease and did not develop GVHD even after CsA was discontinued. The initial CD3$^+$ cell dose was 1 to 3×10^7 cells/kg and the dose was escalated every 4 weeks when the patient did not develop tumor response or GVHD.

Chimerism and Immunologic Analyses

Host-donor cell chimerism after transplantation was analyzed monthly by sex-chromosome fluorescent in situ hybridization or the short tandem repeat method after transplantation (22). The serum anti-carcinoembryonic antigen (CEA) antibody level was determined by enzyme-linked immunosorbent assay as previously described (23). Briefly, 96-well microplates were coated overnight at 4°C with a 5-μg/mL CEA preparation. The plates were washed and blocked for 2 hr at room temperature with 200 μL/well of a 0.1% Tween20, 5% nonfat dry milk, phosphate-buffered saline solution to prevent nonspecific binding. After the plates were washed further, 50 μL of 1:100 diluted patient sera was added per well and incubated for 2 hr at room temperature. After the plates were washed five times, 50 μL of horseradish peroxidase-labeled anti-human immunoglobulin G antiserum at 0.1 μg/mL in blocking buffer was added per well. The plates were incubated for 90 min at room temperature and then washed five times. The conjugated anti-CEA antibody was detected by adding 100 μL of tetramethylbenzidine substrate per well, incubating for 30 min at room temperature, adding 50 μL of 2N H_2SO_4 per well to terminate the reaction, and measuring the absorbance at 450 nm. The net anti-CEA antibody absorbance was determined by subtracting the absorbance of a noncoated well from the gross absorbance.

Outcome Measures

The primary endpoint of this study was transplant-related mortality within 100 days after transplantation. The secondary endpoint was the tumor response within 6 months after transplantation. Toxicities associated with the conditioning regimen were graded according to the criteria of Bearman et al. (24). Objective tumor response was evaluated by an independent radiologist using a monthly computed tomographic (CT) scan. Complete response was defined as disappearance of all clinical evidence of tumor for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the sum of the products of the maximum diameter and its perpendicular diameter of all measurable lesions for a minimum of 4 weeks (1). The tumor marker response was evaluated by biweekly measurement of the serum CA19-9 level, because imaging modalities including ultrasonography and CT scan are

TABLE 1. Characteristics of the patients

Patient	Age/sex	Prior treatment	Duration from Dx to transplant (mo)	Meta	HLA	No. of CD34$^+$ cells
1	48/M	Gem	3	—	6/6	4.8×10^6/kg
2	40/M	Gem+RT, Gem	9	—	6/6	5.1×10^6/kg
3	57/F	Gem+CDDP	4	Liver	6/6	4.0×10^6/kg
4	36/F	Gem	3	Liver	6/6	2.9×10^6/kg
5	59/M	Gem	2	—	6/6	5.6×10^6/kg
6	66/F	Gem	2	Peritonitis	6/6	2.0×10^6/kg
7	61/M	Gem+RT	12	Liver	6/6	3.0×10^6/kg

Dx, diagnosis; Meta, metastatic lesion; Gem, gemcitabine; RT, local radiation; CDDP, cisplatin.

TABLE 2.   Outcome after RIST

Patient	Donor chimerism (%)	aGVHD	Objective response	Final outcome
1	100	III	MR	Died as a result of bacteremia on day 192
2	100	II	SD	Died as a result of PD on day 293
3	100	II[a]	MR[d]	Died as a result of PD on day 262
4	100	II[a]	PD	Died as a result of PD on day 72
5	100	II	SD[c,d]	Died as a result of PD on day 587
6	100	III[b]	PD	Died as a result of PD on day 229
7	100	III[a]	SD	Died as a result of pneumonia on day 83

[a] GVHD that occurred after the rapid tapering of immunosuppressants.
[b] GVHD that occurred after DLI.
[c] Partial response on tumor marker evaluation.
[d] Morphine was discontinued.
aGVHD, acute graft-versus-host disease; MR, minor response; SD, stable disease; PD, progressive disease; DLI, donor lymphocyte infusion.

not sufficient to determine the accurate tumor size of pancreatic cancer (25). For patients with a normal value of CA19-9 before RIST, CEA, Dupan-II, or Span-I was measured instead. A complete marker response was defined as normalization of the tumor marker for a minimum of 4 weeks. Minor and partial responses were defined as decreases of 25% to 50% and greater than 50%, respectively, in the tumor marker for a minimum of 4 weeks.

FIGURE 1.   (A) Cumulative incidence of grade II to IV acute GVHD (*solid line*) and tumor response including both the objective response and the tumor marker response (*broken line*). (B) Overall survival after RIST against advanced pancreatic cancer.

Statistical Considerations

We defined success as the absence of early transplant-related mortality and planned seven and nine patients in the first and second stages of the study, with target and lower success rates of 80% and 50% and α and β errors of 10% and 10%, respectively (26). This is an analysis of the seven patients in the first stage. The cumulative incidence of tumor response was calculated by Gray's method considering death without response as a competing risk (27).

RESULTS

Patients

In the first stage of this study, seven patients with a median age of 57 years (range, 36–66 years) underwent RIST (Table 1). The duration from diagnosis to transplantation was 2 to 12 months. Four had metastatic disease and three had locally advanced disease. Prior treatment consisted of chemotherapy mainly with gemcitabine without an objective response, except for one patient (patient 2) who achieved a transient partial response after gemcitabine combined with local irradiation. All of the patients had progressive disease just before the conditioning regimen. All received a peripheral blood stem-cell graft from an HLA-identical sibling donor. The median number of CD34$^+$ cells in the graft was 4.0×10^6 cells/kg recipient body weight (range, 2.0–5.6 cells/kg). Three patients (patients 2, 5, and 6) underwent DLI for tumor progression 221, 336, and 69 days after transplantation, respectively. The dose of infused CD3$^+$ cells ranged between 2.7×10^7 and 1.8×10^8 cells/kg.

Regimen-Related Toxicity, Engraftment, and GVHD

Regimen-related toxicities were generally mild and well tolerated. Grade II to IV toxicity according to Bearman's grade was observed in two patients. One developed mild hepatic veno-occlusive disease, which recovered spontaneously. Another developed ileus caused by the pancreatic head tumor during the neutropenic period, which required nasogastric suction.

The median number of infused CD34$^+$ cells was 4.0×10^6 cells/kg (range, 2.0–5.6 $\times 10^6$ cells/kg). Neutrophil engraftment was obtained within 12 days (range, 11–12 days)
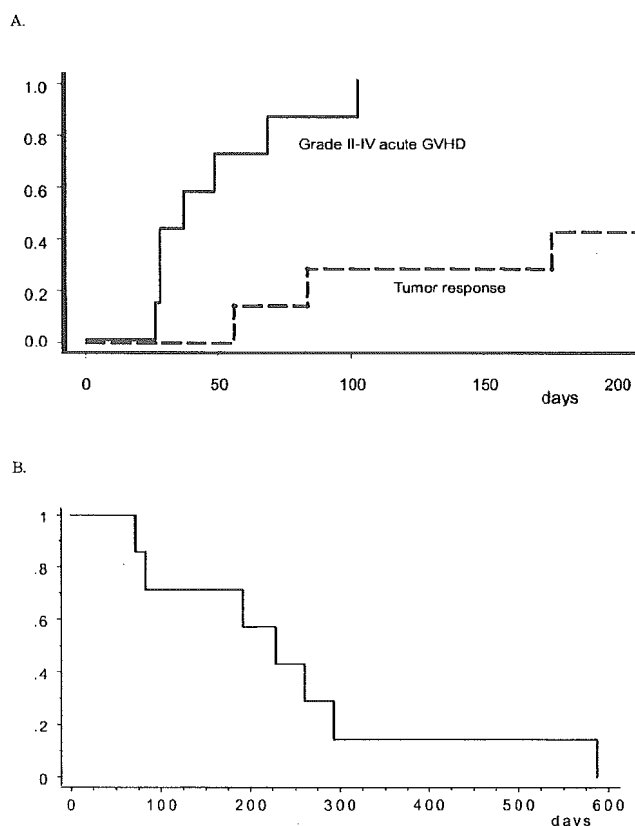
FIGURE 2. Serial changes in the values of tumor markers. CA19-9 was used all except patients 1 and 7, in whom CEA was measured instead because the CA19-9 level was within normal limits before transplantation. The units for the y-axis are units per milliliter for CA19-9 and nanograms per milliliter for CEA. A log scale was used for the y-axis to show serial data of all patients in one figure. Pre, pretransplant level.
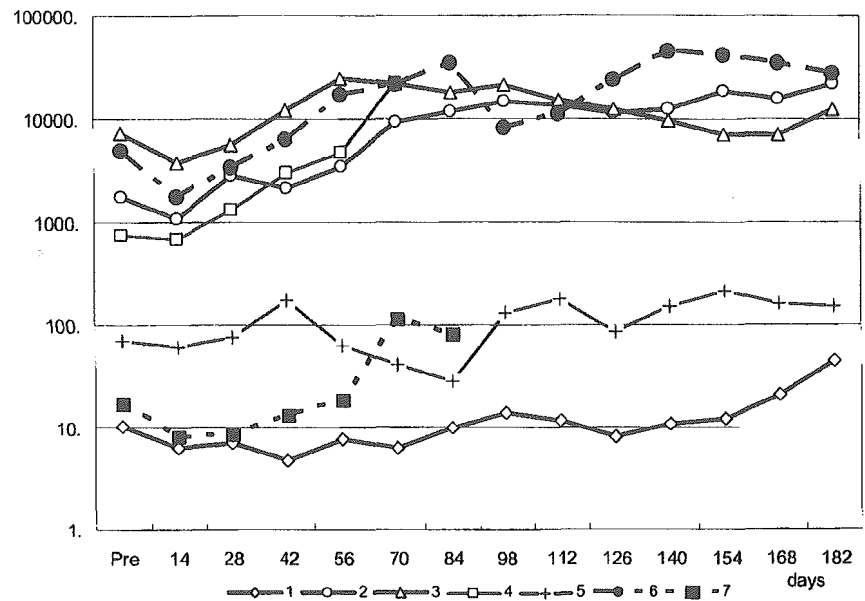
after transplantation. Complete donor chimerism (>95%) was achieved in all patients by day 28 and maintained thereafter (Table 2). Grade II to III acute GVHD was observed in three patients (patients 1, 2, and 5) without rapid tapering of CsA, in three (patients 3, 4, and 7) after rapid tapering of CsA, and in one after DLI (Fig. 1A). Acute GVHD limited to the skin was cured with topical steroid only, whereas gut GVHD was successfully treated with systemic steroid.

Transplant-Related Mortality and Survival

Transplant-related mortality within 100 days after transplantation was observed in 1 patient (patient 7), who died as a result of pneumonia on day 83. Another patient (patient 1) died with bacteremia on day 192 caused by bacterial cholangitis. The other five patients died as a result of progressive disease. Median survival after RIST was 229 days (Fig. 1B), which was longer than the median survival of control patients after they were referred to our hospital (125 days), but this difference was not statistically significant.

Tumor Response

An objective minor response on CT scan was seen in two patients (patients 1 and 3) (Table 2 and Fig. 1A). Another patient (patient 5) achieved a partial tumor marker response. Two (patients 3 and 5) of the responders became free from all analgesics after achieving tumor regression.

As shown in Figure 2, the serum CA19-9 or CEA level generally increased within 6 to 8 weeks after transplantation after a transient decline associated with the conditioning chemotherapy. However, it stabilized (n=1) or began to decrease (n=3) thereafter, associated with the discontinuation of CsA or the development of GVHD. This suggests that the antitumor effect was caused by a GVT effect, not a chemotherapy effect. In particular, the serum CA19-9 level decreased from 25,180 U/mL on day 56 to 7,100 U/mL on day 154, with a tumor shrinkage on CT scan in patient 3 after the development of gut GVHD on day 69 (Fig. 3A). Evidence of a GVT effect against pancreatic cancer was also clearly seen in patient

6, who underwent DLI on day 69 for a progressive peritoneal metastatic lesion. The serum CA19-9 level decreased from 35,160 U/mL to 8,140 U/mL in 1 month, with an improvement of the peritoneal lesion on abdominal CT scan (Fig. 3B). However, these tumor responses were not durable and the response duration was between 28 and 98 days.
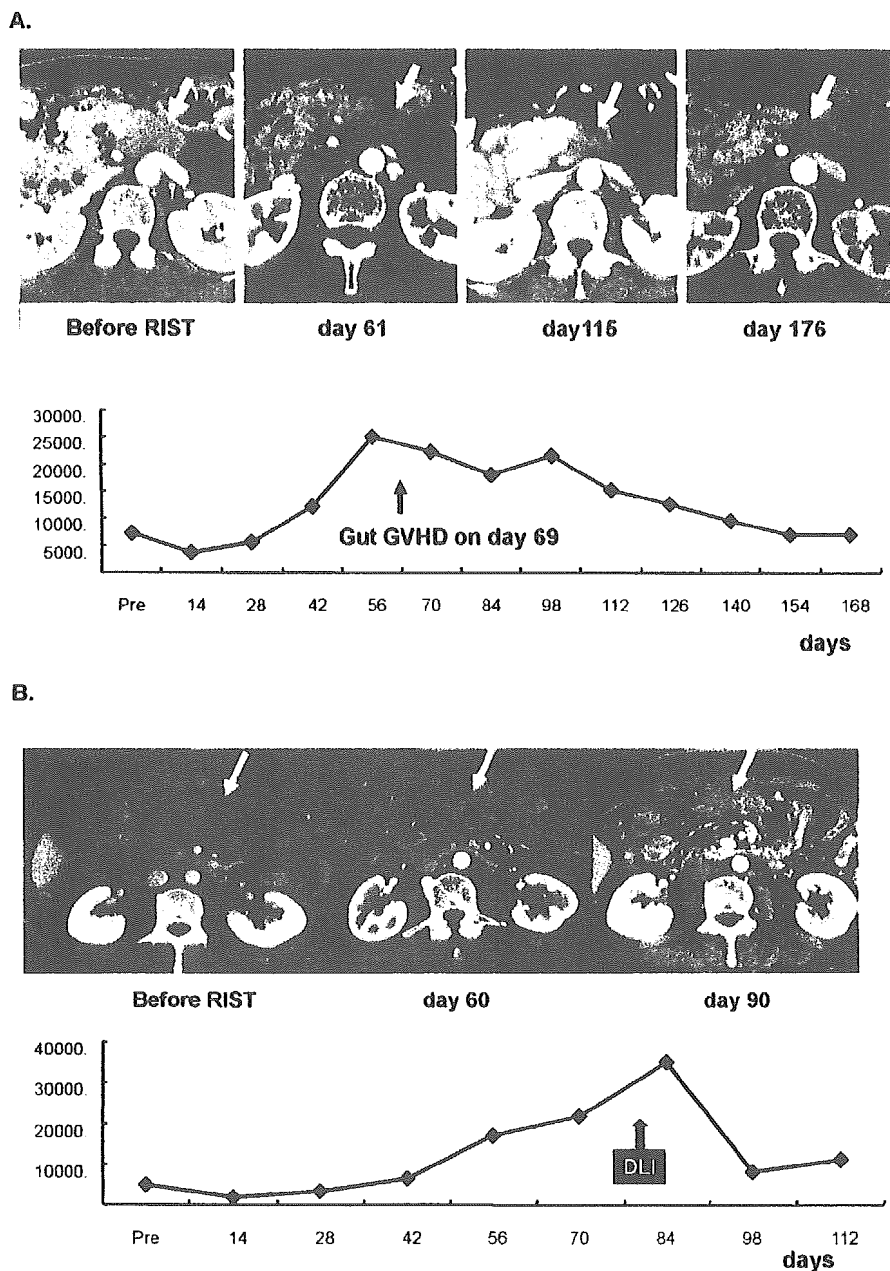
Serum Anti-CEA Antibody Level

Serum anti-CEA antibody levels were sequentially measured before and after RIST. The serum anti-CEA antibody level before RIST was higher than that in a normal population (n=3), with borderline significance (optical density, 0.109±0.065 vs. 0.028±0.030; P=0.076). It generally decreased 1 month after RIST with conditioning chemotherapy. Thereafter, an increase in the serum anti-CEA antibody level was observed in three patients. All three of these patients showed a tumor response, including one after DLI, whereas only one of the four patients without an increase in the serum anti-CEA antibody level showed a response. As shown in Figure 4, the increase in the serum anti-CEA antibody level was simultaneously associated with, or followed by, a decrease in the tumor marker level. However, this response was suppressed by the administration of high-dose steroid (Fig. 4A).

The increase in the serum anti-CEA level did not reflect nonspecific immune recovery, because we did not observe a significant relationship between serum anti-CEA antibody levels and antibody levels against other viral antigens (data not shown). In addition, the increase in the serum anti-CEA level did not result from a decrease in the serum CEA antigen that may absorb anti-CEA antibody, because we did not observe a negative relationship between them (data not shown).

DISCUSSION

In this study, we showed that RIST is a feasible treatment for patients with advanced pancreatic cancer. In addition, an objective response and a tumor marker response were observed in two and one of seven patients, respectively, who

A.

Before RIST   day 61   day116   day 176

Gut GVHD on day 69

Pre 14 28 42 56 70 84 98 112 126 140 154 168

days

B.

FIGURE 3. (A) Serial abdominal CT scans and serum CA19-9 levels of a patient (patient 3) who achieved an objective response after transplantation. The pancreatic body tumor shrunk and the serum CA19-9 level decreased after the development of gut GVHD. (B) Serial abdominal CT scans and serum CA19-9 levels of a patient (patient 6) who achieved an objective response after DLI. The peritoneal lesion worsened in the first 2 months after transplantation. However, the tumor began to shrink and the serum CA19-9 level rapidly decreased after DLI.

Before RIST   day 60   day 90

DLI

Pre 14 28 42 56 70 84 98 112

days

had not responded to conventional treatments. A response to DLI was seen in another patient. Although it is too early to evaluate survival after RIST, the median survival of 229 days might be better than that after conventional treatments. However, the tumor response was not durable, and all of the responding patients eventually died as a result of tumor progression.

It has been shown that at least 2 months are required to obtain a GVT effect after RIST against solid cancers. Childs et al. reported that tumor growth was frequently observed during the first few months after RIST for renal cell cancer (9). Therefore, we added gemcitabine to the combination of fludarabine and busulfan, a widely used conditioning regimen in RIST (4, 17), to suppress tumor progression before the emergence of a GVT effect, because pancreatic

cancer progresses much faster than renal cell cancer. A synergistic antitumor effect with the combination of gemcitabine and fludarabine has been demonstrated in in vitro studies (28).

It can be difficult to distinguish between a chemotherapy effect and a GVT effect after RIST. In this study, however, a transient chemotherapy effect attributable to the conditioning regimen was observed as tumor marker regression during the first 2 weeks after RIST. Thereafter, tumor marker began to increase, which suggested that the chemotherapy effect did not persist for longer than 2 weeks. The second regression of tumor marker was observed at least 2 months after RIST, associated with the discontinuation of CsA, the development of GVHD, or DLI. An objective tumor response was also observed at least 2 months after RIST. Therefore, the GVT effect
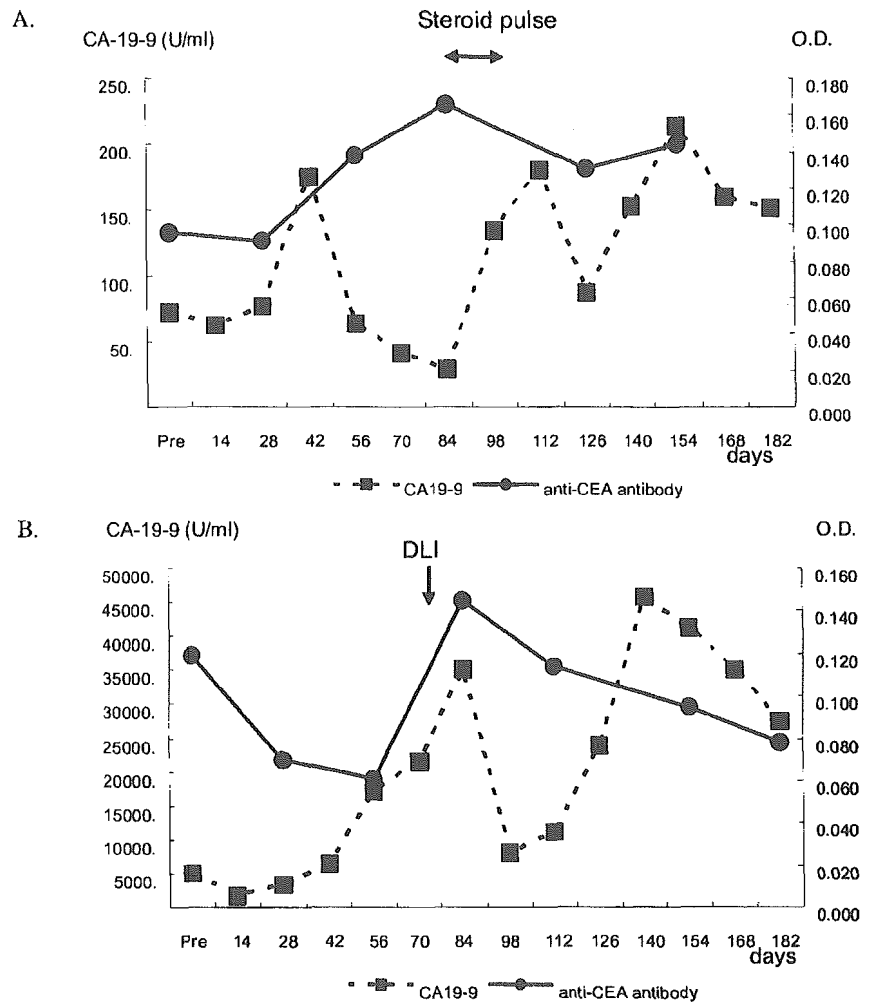
FIGURE 4. Serial changes in the serum CA19-9 level and anti-CEA antibody level. (A) Patient 5, who achieved a partial response on tumor marker evaluation. (B) Patient 6, who showed a tumor response after DLI.

against pancreatic cancer was clearly distinguished from the chemotherapy effect in this study.

Although a GVT effect against pancreatic cancer was seen in this study, this effect did not persist for more than 3 months. In addition, the development of GVHD was required to achieve a GVT effect. Therefore, we need a strategy for maintaining the GVT effect for a longer period without aggravating GVHD. To accomplish this, a specific immunotherapy against pancreatic cancer is required. Candidate target antigens include CA19-9, CA242, CEA, Her-2, mutated K-ras, and MUC-1 (29). Among these, CEA is attractive, because it is expressed in 85% to 90% of pancreatic cancer (29), and a specific immunotherapy against CEA could also be applied to other gastrointestinal cancers. Therefore, we retrospectively measured serial serum anti-CEA antibody levels in these patients. An increase in the serum anti-CEA antibody level after a transient decline just after RIST was associated with a tumor response. An elevation of serum anti-CEA antibody levels has already been demonstrated after a vaccine therapy targeting CEA (23, 30). Also, the presence of anti-CEA antibody at diagnosis was associated with better survival in patients with colon cancer, and thus anti-CEA antibody was suggested to have biologic significance (31). Thus, the GVT effect against pancreatic cancer in this study might be in

part attributable to the specific immunity against CEA antigen. We also tried to detect CEA-specific cytotoxic T cells by enzyme-linked immunospot assay, but failed, probably because of the use of frozen peripheral blood mononuclear cells.

A major difference between RIST for hematologic malignancies and that for pancreatic cancer is the complications after transplantation. One patient (patient 1) experienced repeated episodes of biliary stent obstruction and bacterial cholangitis. Another two patients (patients 2 and 7) developed intestinal obstruction, probably because of the pancreatic head tumor. Therefore, a clinical trial of RIST for pancreatic cancer should be performed in close cooperation with the transplantation staff and gastroenterologists.

CONCLUSION

RIST appeared to be a feasible treatment for patients with advanced pancreatic cancer. The existence of a GVT effect against pancreatic cancer was strongly suggested by this study, but this effect was not durable and required the development of GVHD. We need to refine the strategy, for example, by the combination of specific immunotherapy against CEA after RIST.

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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.

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

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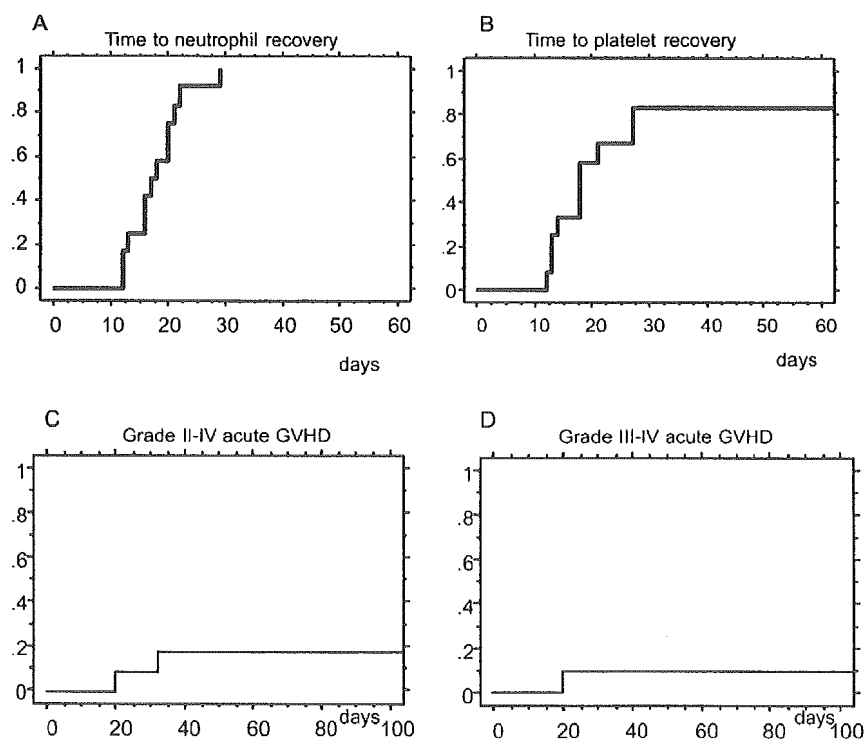
FIGURE 1. Days to neutrophil (A) and platelet (B) recovery and cumulative incidence of grades II to IV (C) and grades III and IV (D) acute GVHD after transplantation.

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
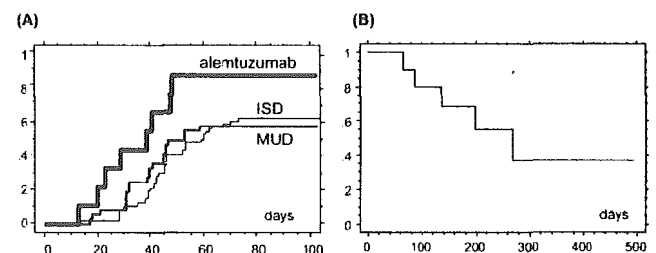
FIGURE 2. Cumulative incidence of cytomegalovirus reactivation detected by antigenemia assay, grouped according to the donor type (A). Overall survival of all patients (B). ISD, human leukocyte antigen (HLA)-identical sibling donors; MUD, matched unrelated donors.
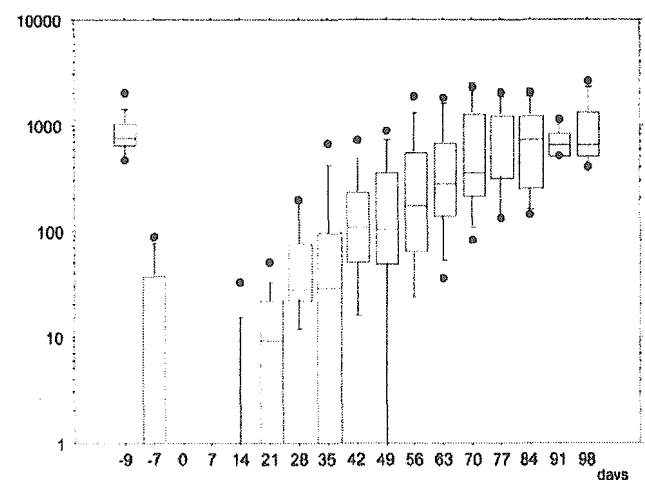
FIGURE 3. Recovery of peripheral blood lymphocytes after transplantation; 10, 25, 50, 75, and 90 percentile values (*box-and-whisker plot*). Outliers (*dots*).

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 pa-

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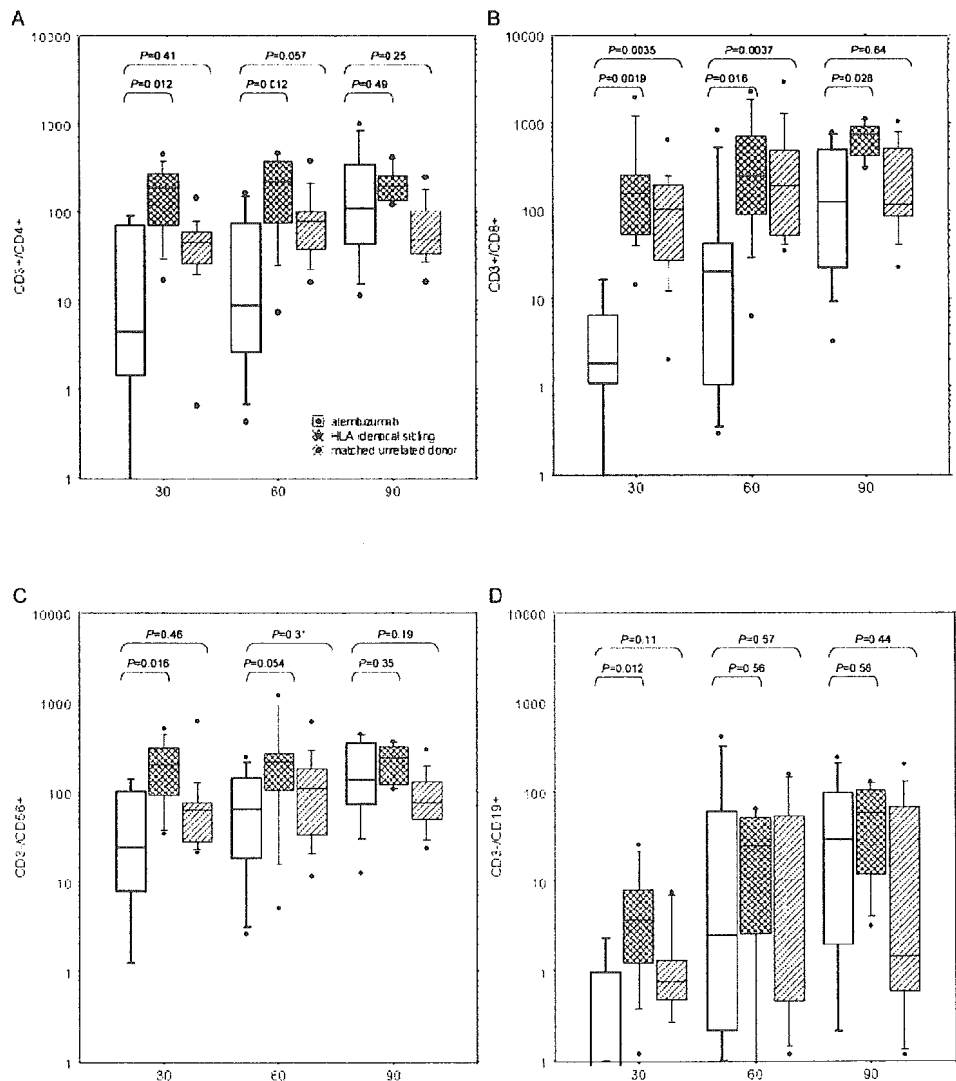
FIGURE 4. Recovery of CD3+/CD4+ (A), CD3+/CD8+ (B), CD3−/CD56+ (C), and CD3−/CD19(+) (D) cells on days 30, 60, and 90 after transplantation, grouped by the donor type; 10, 25, 50, 75, and 90 percentile values (*box-and-whisker plot*). Outliers (*dots*).

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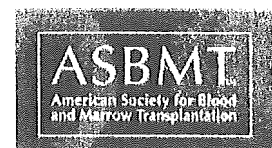
Effect of Conditioning Regimen on the Outcome of Bone Marrow Transplantation from an Unrelated Donor

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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.
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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-

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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 × 10⁸ 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 cate-

gory, 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 × 10⁸ cells/kg	1.00		1.00	
≥3.0 × 10⁸ 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		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