Table 2. Cytokine secretion profile of the generated cells

Cytokine combination (s)	Concentration, pg/mL				
	INF-γ	IL-6	IL-8	IL-10	IL-12p70
TPO	< 5	< 5	302 ± 18	< 5	< 5
TPO + TNF-α	< 5	5.5 ± 0.8	> 5000	< 5	< 5
TNF-α	< 5	7.0 ± 0.8	3152 ± 74	< 5	< 5

Purified human CD34$^+$ cells were cultured for 7 days with or without TPO and TNF-α, and the supernatants were collected. The cytokines secreted during culture were measured using a cytometric beads array system.

vivo and in vitro. As a result, we assessed the cytokine activities in the media of CD34$^+$ cell culture in the presence of TPO and/or TNF-α (Table 2).

TPO alone induced a small amount of IL-8, whereas TNF-α induced the secretion of IL-6 and IL-8 but not that of IL-1β, IL-10, or IL-12p70. The combination of TPO and TNF-α further enhanced IL-8, but not IL-6, secretion. These results suggest that TNF-α is a primary factor to induce the other proinflammatory cytokines and that TPO synergizes with TNF-α for only IL-8 secretion.

Hemophagocytosis by CD11c$^+$ cells in vivo is confirmed

Our observation clearly shows that developing DCs under hematopoietic and inflammatory conditions phagocytose codifferentiated progenitor cells with damage by inflammatory cytokines. We therefore attempted to corroborate that our observation in vitro also takes place in situ. To do so, we stained specimens of bone marrow smears from patients with hemophagocytic syndrome with DC markers, such as CD11c, CD83, CD86, and mannose receptor, and the megakaryocytic marker CD61 or the erythroid marker GPA. As shown in Figure 6, hemophagocytic cells (Figure 6A) were weakly positive for CD83 (Figure 6B), while they were strongly positive for CD86 (Figure 6C), mannose receptor (Figure 6D), and CD11c (Figure 6E-F). Double staining with anti-CD11c and anti-CD61 showed that CD11c$^+$ cells phagocytose platelets (Figure 6E-F,H) and CD61$^+$ substrates (Figure 6G) in vivo. Our previous report demonstrated that nonerythroid cells generated from CD34$^+$ cells treated with erythropoietin, SCF, IL-3, and TNF-α expressed DC phenotype and that CD11c$^+$ DCs phagocytosed immature erythroid cells.[22] We therefore stained bone marrow cells from the patients with CD11c$^+$ and erythroid marker, GPA (Figure 7A-C). As a result, CD11c$^+$ cells in the patients' bone marrow were consistently shown to capture erythroid as well as megakaryocytic cells. In addition, staining with α-naphthyl butyrate (α-NB) of hemophagocytic cells showed a similar perinuclear distribution of granules in immature DCs generated from purified normal human CD34$^+$ cells (Figure 7D-E). Such a distribution pattern of α-NB–positive granules was different from pancellular staining in macrophages.

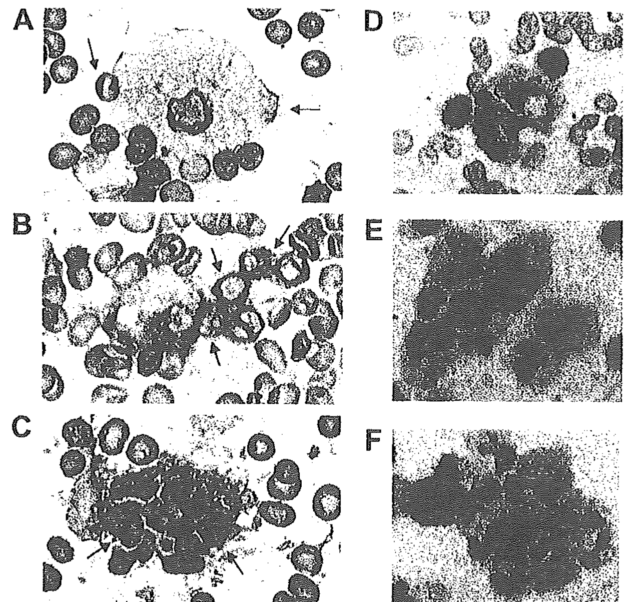

Figure 7. Capture of erythroid progenitor cells expressing GPA by CD11c$^+$ cells from bone marrow of hemophagocytic syndrome. Bone marrow smear preparations from patients with hemophagocytic syndromes were stained with CD11c (red)/GPA (brown) for patient 1 (A) or CD11c (brown)/GPA (red) for patient 2 (B) and patient 3 (C). The arrows indicate GPA-positive erythroid cells. Staining with α-NB of hemophagocytic cells is also shown in patient 3 (D) and DCs generated from purified normal human CD34$^+$ cells in the presence of TPO and TNF-α (day 5 [E]; day 7 [F]).

To obtain more detailed information about hemophagocytic cells in these patients, we clarified the frequency of monocytic cells macrophages by May-Grünwald-Giemsa staining and CD11c$^+$ cells associated with/capturing dying hematopoietic progenitor cells by immunohistochemical staining (Table 3). The frequencies of hemophagocytic cells

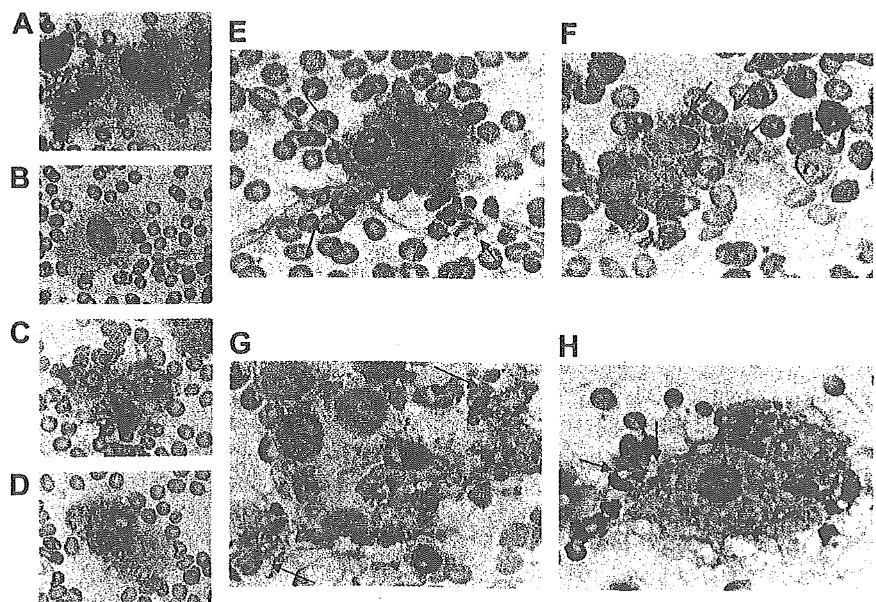

Figure 6. Hemophagocytosis by CD11c$^+$ cells in bone marrow cells from patients with hemophagocytic syndrome. Bone marrow smear preparations from patients with hemophagocytic syndrome were stained with May-Grünwald-Giemsa staining (patient 1) (A), CD83 staining (patient 1) (B), CD86 staining (patient 1) (C), mannose receptor staining (patient 1) (D), or CD11c (brown)/CD61 (red) double staining for patient 1 (E), patient 2 (F-G), and patient 3 (H). The arrows indicate CD61$^+$ platelets or substrates.

Table 3. Frequencies of CD11c+ hemophagocytic cells in the bone marrow of patients with hemophagocytic syndrome

	Patient no.		
	1	2	3
Cellularity	Hypo	Normo	Hypo
Frequencies, %			
By May-Grünwald-Giemsa staining			
Monocytic cells/macrophages in total bone marrow	35.8	15.0	26.0
Hemophagocytes in total bone marrow	13.6	3.8	5.2
CD11c+ cells by immunohistochemical staining			
In total bone marrow	31.0	11.6	27.8
In hemophagocytes	84.4	62.2	70.3
Associated with/capturing CD61+ cells/substrates	36.1	42.0	9.7
Associated with/capturing GPA+ cells/substrates	94.4	53.6	91.4

GPA indicates glycophorin A; hypo, hypocellular marrow; normo, normocellular marrow.

were in the range of 3.8% ± 13.6% of total bone marrow cells, which consisted of 35.8% ± 15.0% of monocytic cells/macrophages and 31.0% ± 11.6% of $CD11c^+$ cells. In addition, $CD11c^+$ cells were composed of 84.4% ± 62.2% of hemophagocytic cells, and they captured megakaryocytic as well as erythroid cells. These data strongly suggest the possibility that hemophagocytic cells in the bone marrow of patients, at least in part, are DCs.

Discussion

This study demonstrated that TNF-α inhibits the generation of megakaryocytic progenitor cells from human $CD34^+$ cells in the presence of TPO and inversely increases nonmegakaryocytic cells with feature of DCs. These cells expressed the typical surface markers of DCs, such as CD11c, CD4, and CD86. They were closely associated with codeveloping immature megakaryocytic progenitor cells and then captured them. Interestingly, the cells thus generated were capable of inducing autologous MLRs. Like DCs generated by TPO and TNF-α, capture of $CD61^+$ cells by $CD11c^+$ cells in bone marrow was also observed in patients with hemophagocytic syndrome, thus indicating that similar phenomena can take place in vivo. These findings suggest that megakaryocytic and inflammatory costimuli on hematopoietic stem/progenitor cells may induce hemophagocytosis of megakaryocytic cells in a physiologic situation.

We documented that TNF-α inhibits the generation of megakaryocytic progenitor cells from human $CD34^+$ cells in the presence of TPO in a dose-dependent fashion with a half-maximal dose ranging from 0.25 to 2.5 ng/mL. Although considerable advances have been made during the past 2 decades in our understanding of the biology and the clinical rôle of the TNF superfamily, the role of TNF-α in megakaryopoiesis remains controversial. TNF-α stimulates colony formation by a megakaryoblastic leukemia cell line (CMK) established from a patient with Down syndrome,[33] and it also induces megakaryocytic differentiation of the HIMeg-1 cells,[34] a cell line derived from a patient with chronic myeloid leukemia capable of monocytic and megakaryocytic differentiation. In contrast, TNF-α has been well documented to inhibit the megakaryopoiesis of normal progenitor cells. Lu et al[35] reported that TNF-α suppressed colony-forming-unit megakaryocytes derived from human $CD34^+$ cells in the presence of IL-11, IL-3, SCF, and TPO. TNF-α almost completely abrogated the growth of human $CD34^+$ $CD38^-$ progenitor cells in response to TPO alone as well as SCF/FLT3/TPO.[36] It is very likely that the differences between various cell lines and primary cells account for some of the discrepancy between observations made by different researchers. The mechanism by which TNF-α inhibits normal megakaryocytic differentiation remains unclear.

Although TPO has been shown to be the primary regulator of platelet production,[13] many studies have demonstrated that TPO supports the proliferation and long-term expansion of primitive $CD34^+$ cells in synergy with FLT3-L, SCF, and/or IL-3.[15-17] We further demonstrated that TPO and TNF-α permit $CD34^+$ cells to differentiate into $CD4^+$ $CD11c^+$ $CD123^+$ DCs and $CD4^+$ $CD11c^-$ $CD123^+$ cells. The finding that TPO alone did not induce the generation of $CD11c^+$ immature DCs suggests that TNF-α thus plays a critical role in synergy with TPO. The coexpression of TNFR-II and c-mpl on $CD11c^+$ cells suggests that TPO and TNF-α act in synergy with the downstream signaling pathways of both receptors.

In suspension cultures, a rapid increase in the proportion of $CD11c^+$, $CD83^+$, or $CD86^+$ cells was observed as early as day 5. Although $CD34^+$ cells can also differentiate into monocytes/macrophages, it takes 10 to 14 days in vitro.[37] Day 5 $CD11c^+$ DCs were capable of capturing codeveloping megakaryocytic progenitors. Most $CD11c^+$ cells coexpressed CD4 and CD123. Therefore, the $CD4^+$ $CD11c^+$ $CD123^+$ cells are most likely DCs in an immature stage. The remaining nonmegakaryocytic cells consisted of $CD4^+$ $CD11c^-$ $CD123^+$ cells. However, a dot plot analysis showed a continuous CD11c expression in $CD123^+$ cells on day 5 (Figure 2). The proportion of $CD11c^+$ $CD4^+$ cells reached a plateau by day 5, while that of $CD123^+$ cells gradually decreased by day 7 to the equivalent with $CD11c^+$ $CD4^+$ cells. Therefore, some $CD11c^-$ $CD123^+$ cells may not be in a transitional stage to $CD11c^+$ $CD123^+$ cells. Blom et al[28] have shown that $CD34^+$ $CD45RA^+$ $CD123^+$ cells develop into mature DCs in cultures with SCF or FLT3-L through the differentiation pathway of plasmacytoid DC ($CD11c^-$ $CD123^+$) precursor cells. More recently, Chen et al[20] showed that TPO cooperates with FLT3-L in the generation of plasmacytoid DC precursors from human $CD34^+$ cells. DCs derived from plasmacytoid precursor cells are known to lack CD11c and CD1a.[38] The possibility that $CD11c^-$ $CD123^+$ cells are capable of differentiating plasmacytoid DCs upon transfer to the culture with FLT3-L and TPO remains to be elucidated. However, the $CD4^+$ $CD11c^+$ $CD123^+$ DCs are possibly myeloid lineage DCs, because they do not produce a large amount of IFN-α in response to CpG-ODN (data not shown).

The potent effects of TNF-α on generation of DCs and, in turn, the inhibition of TPO-induced megakaryopoiesis are also of particular interest, because the $CD11c^+$ DCs closely associated with codeveloping megakaryocytic progenitor cells and also phagocytosed them (Figure 4). Moreover, those DCs induced autologous MLRs, whereas $CD11c^+$ cells generated by TNF-α alone did not (Figure 5). As previously reported,[39,40] phagocytosis of necrotic but not apoptotic cells induces DC maturation. Under in vitro culture conditions, developing DCs possibly captured both apoptotic and necrotic cells, thus leading to DC maturation. Indeed, the proportions of $CD11c^+$ cells were comparable with each other, but a slight increase in the expression of costimulatory molecules was noted in DCs induced by TPO plus TNF-α. However, DCs generated with TNF-α alone and TNF-α and TPO showed comparable amounts of IL-6 and IL-8, although the latter produced more IL-8 than the former (Table 2).

An infection with a virus, such as Epstein-Barr virus,[41] mumps virus,[42] dengue virus,[43] and hepatitis A virus,[44] has been suggested to result in fatal hemophagocytic syndrome, which is associated

with an overproduction of IFN-γ, TNF-α, IL-1, and IL-6,[41,45] which are known to be potent activators of macrophages. Well-differentiated macrophages have been observed to phagocytose hematopoietic cells in the bone marrow.[46] We herein also show that CD11c$^+$ CD83$^+$ CD86$^+$ cells in patients with hemophagocytic syndrome contained CD61$^+$ cells and their fragments and GPA-positive cells intracellularly (Figures 6-7; Table 3), thus suggesting the involvement of DCs in hemophagocytosis.

Recent studies indicate that DCs are involved in the counterregulation of potential autoimmune T-cell responses[47] and that CD11c$^+$ DCs that have not been activated by pathogen-related or endogenous inflammatory stimuli can significantly contribute to peripheral tolerance by inducing the inactivation and/or deletion of specific T cells.[48] On the other hand, DCs expressing endogenous self-peptides or pulsed ex vivo with immunogenic self-peptides can induce severe autoimmune disease.[47,49] The data presented in this work demonstrate that TNF-α has strong effects on cytokine secretion by developing cells, most probably immature DCs, including the up-regulation of early proinflammatory cytokines (IL-6 and IL-8). On the other hand, TNF-α–treated DCs have been reported to be semimature and induced regulatory cells upon inoculation in vivo.[50,51] Therefore, limited immune response or induction of regulatory T cells may prevent T cell–mediated autoimmune diseases in a steady state as well as in pathologic situations.

In conclusion, this is the first report showing that in the presence of TNF-α the nonmegakaryocytic cells with typical feature of DCs are cogenerated from human CD34$^+$ cells during megakaryocytic differentiation by TPO. The CD4$^+$ CD11c$^+$ CD123$^+$ DCs are physically associated with and phagocytose either developing or dying immature megakaryocytic cells. A similar phenomenon showing engulfment of CD61$^+$ fragment by CD11c$^+$ cells was also observed in the bone marrow cells from patients with hemophagocytic syndrome. Therefore, it may be conceivable that DCs with phagocytic activity during the development in bone marrow may play a crucial role in the maintenance of tolerance for self-substances derived from hematopoietic progenitor cells.

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CASE REPORT

HLA-haploidentical nonmyeloablative stem cell transplantation: induction to tolerance without passing through mixed chimaerism

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Summary There are few reports of unmanipulated HLA-haploidentical nonmyeloablative stem cell transplantation (NST) using only pharmacological acute graft-*vs.*-host disease (GVHD) prophylaxis. We present here a successful case of unmanipulated HLA-haploidentical NST for mediastinal large B cell lymphoma that was resistant to autologous peripheral blood stem cell transplantation (PBSCT). The conditioning regimen consisted of fludarabine, busulfan and rabbit anti-T-lymphocyte globulin (ATG) in addition to rituximab. GVHD prophylaxis was performed using tacrolimus and methylprednisolone 1 mg/kg. The patient had rapid engraftment, with 100% donor chimaerism in the lineages of both T cells and granulocytes on day +12, but developed no GVHD clinically. The patient is still in complete remission past day +1020, with no sign of chronic GVHD without receiving immunosuppressive agents. HLA-haploidentical NST may be performed without utilizing mixed chimaerism.

Keywords HLA-haploidentical nonmyeloablative stem cell transplantation, graft-*vs.*-host disease, graft-*vs.*-lymphoma effect, non-Hodgkin's lymphoma

Introduction

There are only a few reports describing nonmyeloablative stem cell transplantation (NST) from human leucocyte antigen (HLA)-haploidentical donors (Sykes *et al.*, 1999; O'Donnell *et al.*, 2002). We recently showed that the combination of fludarabine, busulfan and anti-T-lymphocyte globulin (ATG), a reduced-intensity regimen (Slavin *et al.*, 1998), enabled engraftment of HLA-haploidentical related transplants (one antigen-mismatch in the graft-*vs.*-host (GVH) direction) (Tamaki *et al.*, 2003). However, in that study, acute graft-*vs.*-host disease (GVHD) could not be sufficiently controlled using GVHD prophylaxis with cyclosporine or tacrolimus (FK506) with or without mycophenolate mofetil. On the contrary, a protocol for HLA-haploidentical NST from 2 to 3 antigen-mismatched donors in the GVH direction without T-cell depletion using more intensified GVHD prophylaxis [FK506 + methylprednisolone (mPSL)] is now being tested in an ongoing study. Among the patients in that ongoing study, we recently encountered a patient with mediastinal large B-cell lymphoma (MLBCL) that was resistant to

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autologous peripheral blood stem cell transplantation (PBSCT), who was successfully treated by HLA-haploidentical NST, and is still in complete remission past day +1020 without receiving immunosuppressive agents.

Case report

A 26-year-old female developed bulky mediastinal and lung masses in May 2000 and was diagnosed with MLBCL. After she received three courses of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), left upper lobectomy was performed for the residual lung mass. Four months later, lymphoma relapsed at the original mediastinal lesion. The patient received two additional courses of CHOP and four courses of ProMACE-CytaBOM (cyclophosphamide, doxorubicin, etoposide, cytaribine, bleomycin, vincristine, methotrexate with leucovorin, prednisone) with some effect, but the lymphoma soon began to grow again. Therefore, autologous PBSCT was performed using a preconditioning regimen consisting of cyclophosphamide, etoposide and ranimustine, but residual tumours remained in the left lung field and in the anterior mediastinum.

As there was no time to locate an unrelated donor, we decided to perform stem cell transplantation (SCT) from her sister with HLA-2-antigen mismatching in both the GVH and host-*vs.*-graft (HVG) directions (patient A24 B61 DRB1 0901/A24 B48 DRB1 0405, donor A2 B54 DRB1 0405/A24 B48 DRB1 0405). Institutional review board approval was obtained for the treatment protocol, and written informed consent was obtained from the patient and her family members, including the donor.

As the patient had already been heavily treated with chemotherapeutic drugs in addition to autologous PBSCT, a reduced intensity regimen was used. Clinical course of the patient was given in Figure 1. The preparative regimen consisted of fludarabine 30 mg/m^2 × 6 on days −10 to −5, oral busulfan 4 mg/kg on days −6 and −5, and rabbit ATG (Fresenius, Gräfelfing, Germany) 1.5 mg/kg × 4 on days −4 to −1. Rituximab 375 mg/m^2 was given on days −12, −5, +2 and +37. GVHD prophylaxis was performed by treatment with FK506 (the target blood concentration was 10–15 ng/ml) from day −3 and with mPSL 1 mg/kg starting from day 11. The patient underwent transplantation of peripheral blood stem cells containing 22 × 10^6 CD34$^+$ cells/kg without any manipulation. Granulocyte colony-stimulating factor 10 μg/kg/day was given from day +8. Haematopoietic reconstitution was rapid, with absolute neutrophil count >0.5 × 10^9/l on day +11 and platelet count >50 × 10^9/l on day +12. On day +12, complete donor chimaerism was confirmed in the cell lineages of both CD3$^+$ cells and granulocytes in the peripheral blood. The patient had no acute or chronic GVHD. The patient had no other post-transplant complications other than haemorrhagic cystitis, which improved after conventional treatments. Chest computed tomography scans performed on day +118 revealed that residual tumours present before NST had disappeared. On day +1020, the patient was still in complete remission with no sign of chronic GVHD without receiving immunosuppressive agents.

Discussion

To date, only a few studies of unmanipulated NST from HLA-haploidentical (mismatch of >1 antigen) donors have been reported. Sykes and colleagues performed transplants in four patients with refractory non-Hodgkin's lymphoma

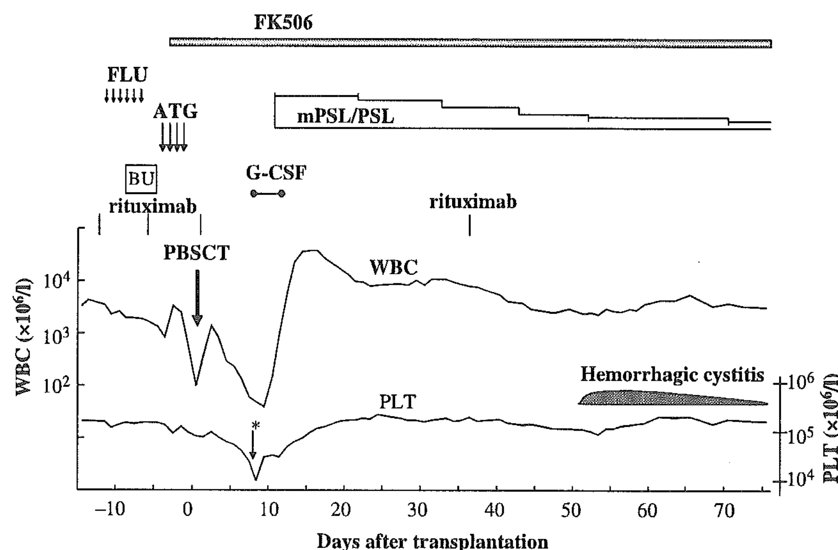

Figure 1. Clinical course of the patient. The bold and thin lines represent the white blood cell (WBC) and platelet (PLT) counts respectively. *The arrow denotes platelet transfusion, which was needed only once. mPSL, methylprednisolone; PSL, prednisone; FK506, tacrolimus; FLU, fludarabine; BU, busulfan; ATG, anti-T-lymphocyte globulin; PBSCT, peripheral blood stem cell transplantation.

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using a reduced conditioning regimen (Sykes *et al.*, 1999). They focused on mixed lymphohaemopoietic chimaerism that was achieved across HLA barriers by a combination of CPA, thymic irradiation and ATG, a conditioning that they expected to specifically target host immune resistance. All patients achieved mixed chimaerism, but all except one, who was alive in complete remission on day +460, died of transplant-related toxicity or progressive disease within a short period of time after transplantation. O'Donnell and colleagues reported 10 patients who underwent HLA 2- or 3-antigen-mismatched BMT with a nonmyeloablative regimen including fludarabine, total body irradiation (2 Gy) and post-transplantation CPA (O'Donnell *et al.*, 2002). Their protocol was characterized by the use of high-dose CPA early after BMT, which was expected to attenuate both the GVH and HVG reactions. Mixed lymphohaemopoietic chimaerism was achieved after transplantation in their study; half of the patients, however, had graft rejection.

The investigators in those studies aimed to utilize mixed chimaerism for achieving bi-directional (GVH and HVG) tolerance. In contrast, the case in the present report suggests that both of these barriers can be overcome by the use of a conventional combination of preparative regimen and pharmacological GVHD prophylaxis even if the patient rapidly achieves 100% donor chimaerism. This rapid engraftment may have been caused by the use of PBSCs in our case (*vs.* the use of BM in studies cited above), in addition to sufficient immunosuppression brought about by the combination of fludarabine, busulfan and ATG.

At present, we do not know which is better, the achievement of rapid donor engraftment or mixed chimaerism in HLA-haploidentical NST settings. However, this successful case suggests that the GVH and HVG barriers in HLA-haploidentical transplantations can be overcome using a reduced preconditioning regimen (fludarabine + busulfan + ATG) and appropriate pharmacological GVHD prophylaxis. However, the feasibility of this transplant strategy must be investigated in a large-scale study.

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Reduced-Intensity Conditioning Followed by Unrelated Umbilical Cord Blood Transplantation for Advanced Hematologic Malignancies: Rapid Engraftment in Bone Marrow

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Abstract

Reduced-intensity (RI) conditioning followed by cord blood transplantation (CBT) is a new treatment modality, but failure to engraft is a major concern. We describe 12 patients with advanced hematologic malignancies who underwent RI conditioning and CBT with a conditioning regimen consisting of 200 mg/m^2 fludarabine (Flu), 50 mg/kg cyclophosphamide (CY), and 3 Gy total body irradiation (TBI). Cyclosporin A and/or methotrexate were used for graft-versus-host disease prophylaxis. Cord blood grafts were not mismatched for more than 2 serologically defined HLA alleles but were later found by high-resolution DNA typing to be mismatched for 2 to 4 alleles in most cases. Short tandem repeat analysis of bone marrow cells at day 14 showed complete donor chimerism in 6 of the patients and mixed chimerism in 5, indicating rapid engraftment in the bone marrow, whereas the remaining patient experienced graft rejection. Neutrophil recovery was achieved at a median of day 17 (range, days 11-24) in 10 of the 11 patients with marrow chimerism at day 14. Of these 10 patients, however, transplantation-related mortality within 100 days occurred in 4 patients who showed failed platelet recovery and a lack of durable engraftment. Overall survival and disease-free survival rates were 41.7% and 33.3%, respectively. These results show that CB mismatched at 2 to 4 HLA alleles and transplanted with the Flu/CY/3 Gy TBI regimen is able to engraft in the bone marrow as early as day 14.
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Key words: Hematologic malignancy; Cord blood transplantation; Adult; Reduced-intensity conditioning

1. Introduction

Allogeneic hematopoietic stem cell transplantation (SCT) is a potentially curative treatment modality for patients with hematologic malignancies [1]. Much of the therapeutic potential of SCT relates to the graft-versus-tumor (GVT) effect, in which donor T-cells mediate the eradication of the host malignancy [2]. Conventional myeloablative conditioning regimens, including high-dose chemotherapy and lethal total body irradiation (TBI), usually exert antitumor effects as well, and the intensive immunosuppression of these regimens allows the establishment of complete donor chimerism. However, these regimens are often accompanied by significant side effects, termed *regimen-related toxicity* (RRT) [3]. To minimize RRT, investigators have explored reduced-intensity (RI) preparative regimens for older patients or for patients with a poor performance status, organ dysfunction, or extensive prior therapy [4-6]. Grafting with RI conditioning regimens still facilitates a GVT effect.

Umbilical cord blood (CB) is increasingly being used as a source of stem cells for SCT as an alternative to bone marrow or peripheral blood stem cells harvested from human leukocyte antigen (HLA)-matched siblings or unrelated donors [7-8]. Advantages of CB as donor cells include the immediate availability of cryopreserved cells, less strict requirements for HLA matching between donor and recipient, and a low risk of inducing severe graft-versus-host dis-

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ease (GVHD). However, a major disadvantage of CB transplantation (CBT) is the availability of only a limited number of cells from a single CB donor, often resulting in graft failure and poor survival prospects for adult patients.

The combined use of RI regimens and CBT (RI-CBT) represents a new treatment modality for hematologic malignancies in patients without suitable marrow donors or patients otherwise not eligible for myeloablative SCT [9]. However, there are few reports thus far on RI-CBT for adult patients [10-12], and the optimal RI preparative regimen for CBT has not been firmly established. A major problem to be addressed in RI-CBT is graft failure. Barker et al [10] reported that a regimen of fludarabine (Flu), cyclophosphamide (CY), and 2 Gy of total body irradiation (TBI) (Flu/CY/2 Gy TBI regimen) enabled rapid and efficient engraftment of donor cells in RI-CBT. Here we report the results of the use of RI-CBT with a Flu/CY/3 Gy TBI conditioning regimen to treat 12 patients with advanced hematologic malignancies.

2. Patients and Methods

2.1. Patient Eligibility

Patients enrolled in this study had organ dysfunction, extensive prior chemotherapy, or a poor performance status. These patients did not have a related donor matched at 6 of 6 or 5 of 6 HLA alleles or an unrelated donor matched at 6 of 6 HLA alleles, or they needed urgent SCT within 3 months. All patients provided written informed consent. This pilot study was approved by the Institutional Review Board of Hyogo College of Medicine.

2.2. CB Grafts

CB was sought through the Japan Cord Blood Bank Network, which consists of 11 local cord blood banks in Japan [13]. A CB graft was selected on the basis of serologic matching for 4 to 6 alleles at 3 HLA loci (class I HLA-A and HLA-B, and class II HLA-DR), as determined by a standard complement-dependent microlymphocytotoxicity technique [14]. Selected CB grafts had a cryopreserved cell dose of at least 2×10^7 nucleated cells/kg recipient body weight. When multiple suitable CB units were available, we selected the unit that displayed the highest nucleated cell dose from the units that had no more than 2 HLA mismatches. Prior to shipping from the local cord blood bank, confirmatory high-resolution DNA typing of class I HLA-A and HLA-B alleles and class II DRB1 alleles was performed by means of polymerase chain reaction (PCR) analysis with sequence-specific primers [15], PCR analysis with sequence-specific oligonucleotide probes [16], or PCR-based single-strand conformation polymorphism analysis [17]. All of the CB aliquots used were single units and not depleted of T-lymphocytes.

2.3. Preparative Regimen

The preconditioning regimen (the Flu/CY/3 Gy TBI regimen) consisted of 50 mg/kg CY on day –6, 40 mg/m^2 Flu daily for 5 days from days –6 to –2, and 3 Gy TBI (in 2 fractions on day –1). GVHD prophylaxis consisted of a continuous infusion of 3 mg/kg cyclosporin A (CsA) from day –3 until the patients tolerated oral administration. After neutrophil engraftment and in the absence of acute GVHD, CsA was tapered 10% per week starting at approximately day 35. This early tapering of CsA treatment was intended to induce a graft-versus-leukemia effect through attenuated GVHD prophylaxis [18]. Although short-term methotrexate treatment was administered at 10 mg/m^2 (day 1) and 7 mg/m^2 (days 3 and 6) in the first 3 patients, it was omitted in subsequent patients in this context. Granulocyte colony-stimulating factor was administered to all patients at 5 μg/kg per day from day 1 until neutrophil recovery.

2.4. Donor Chimerism Analysis

Donor chimerism was analyzed in marrow and/or blood samples on days 14, 21, 35, and 100, or as clinically dictated. Chimerism was determined by quantitative PCR analysis of informative short tandem repeat (STR) regions in the recipients and donors (STR-PCR) [19]. DNA was extracted from bone marrow or blood cells by means of a SepaGene DNA isolation kit (Sanko Junyaku Co, Tokyo, Japan) and amplified with fluorescent PCR primers (AmpFLSTR Profiler PCR amplification kit; Applied Biosystems, San Jose, CA, USA). The fluorescent PCR products were separated by capillary electrophoresis using a 310 Genetic Analyzer (Applied Biosystems). GeneScan and GeneMapper software packages (Applied Biosystems) were used to calculate the percentages of donor and recipient DNA. Complete (donor) chimerism was defined as the detection of 100% donor DNA in a sample.

2.5. Engraftment

Engraftment was defined when white blood cell counts $>1.0 \times 10^9$/L or absolute neutrophil counts $>0.5 \times 10^9$/L were obtained for 3 consecutive days after transplantation and the attainment of these count thresholds was accompanied by the detection of donor chimerism. Bone marrow engraftment was defined as donor chimerism in the bone marrow detected by STR-PCR analysis, even if the peripheral blood cell count did not reach the engraftment level described above. Primary graft failure (rejection) was defined as peripheral and marrow hypoplasia occurring after transplantation without the detection of donor markers by cytogenetic and/or molecular techniques and was estimated at approximately day 30.

2.6. Graft-versus-Host Disease

Acute GVHD was clinically diagnosed by means of the criteria described by Glucksberg et al [20]. Patients who survived >100 days were evaluated for chronic GVHD.

2.7. RRT and Transplantation-Related Mortality

RRT was defined as any nonhematologic organ dysfunction from day 0 to day 28 [3]. Transplantation-related mortality (TRM) was defined as death without progression of the primary disease.

Table 1.
Characteristics of Patients*

Patient No.	Age, y/Sex	Weight, kg	Disease	Status at Transplantation	HLA Disparity† Serology	HLA Disparity† DNA Typing	GVHD Prophylaxis	NC, $\times 10^7$/kg	CD34, $\times 10^6$/kg
1	40/F	57	NHL-REL	Refractory‡	5/6 (B)	4/6 (A, B)	CsA/MTX	2.26	0.59
2	49/F	54	AML-CR	CR§	4/6 (A, A)	3/6 (A, A, DR)	CsA/MTX	2.26	0.74
3	35/M	58	AML-CR	CR§	4/6 (B, DR)	4/6 (B, DR)	CsA/MTX	2.29	0.80
4	23/F	53	AML-REL2	Refractory§	4/6 (B, DR)	4/6 (B, DR)	CsA	2.35	0.87
5	34/M	67	NHL-REL2	Refractory‡	4/6 (B, DR)	4/6 (B, DR)	CsA	2.52	0.87
6	55/F	48	ATL-REL	Refractory	4/6 (A, DR)	3/6 (A, DR, DR)	CsA	2.55	0.91
7	55/F	62	MDS-REL2	Refractory	6/6	6/6	CsA	2.60	0.91
8	19/M	61	ALL-REL	Refractory§	4/6 (B, DR)	4/6 (B, DR)	CsA	2.63	0.92
9	56/M	57	MDS-REL	Refractory	4/6 (B, DR)	3/6 (A, B, DR)	CsA	2.64	0.97
10	63/M	72	MDS-REL	Refractory	5/6 (B)	4/6 (B, DR)	CsA	2.71	0.98
11	55/M	63	ATL-REL1	Refractory	5/6 (DR)	3/6 (A, B, DR)	CsA	2.85	1.27
12	62/M	61	MDS-REL	Refractory	4/6 (B, DR)	2/6 (A, B, DR, DR)	CsA	3.33	2.04

*GVHD indicates graft-versus-host disease; NC, nucleated cells; CD34, CD34+ cells; NHL, non-Hodgkin's lymphoma; REL, relapse; CsA, cyclosporin A; MTX, methotrexate; AML, acute myelogenous leukemia; CR, complete remission; ATL, adult T-cell leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia.

†HLA disparities between donor and recipient are shown in parentheses.

‡Patient who had undergone previous radiation therapy.

§Patient who had undergone previous allogeneic stem cell transplantation.

3. Results

3.1. Patient Characteristics

Twelve patients underwent RI-CBT between August 2003 and July 2004 at our institution. The patients' characteristics are presented in Table 1. The diagnoses included acute myelogenous leukemia (n = 3), acute lymphoblastic leukemia (n = 1), non-Hodgkin's lymphoma (n = 2), myelodysplastic syndrome (n = 4), and adult T-cell leukemia (n = 2). Ten (83%) of the 12 patients had refractory or relapsed disease, indicating that they had a poor prognosis. The other 2 patients had relapsed after their first allogeneic SCT and then achieved advanced complete remission. The median age of the patients was 49 years (range, 19-63 years), and the median weight was 58 kg (range, 48-72 kg).

3.2. Graft Characteristics

The median number of nucleated cells infused was 2.55×10^7/kg body weight (range, $2.26\text{-}3.33 \times 10^7$/kg), and the median number of infused CD34+ cells was 0.91×10^5/kg body weight (range, $0.59\text{-}2.04 \times 10^5$/kg) (Table 1). Assessment of HLA disparity using both standard serologic methods and high-resolution DNA typing is shown in Table 1. We originally selected CB mismatched for no more than 2 serologically defined HLA alleles. However, DNA analysis revealed 1 or 2 additional HLA mismatches in 7 of the 12 patients. Therefore, 92% of the CB grafts in fact were HLA mismatched for 2 to 4 alleles.

3.3. Engraftment and Chimerism

The clinical outcomes for all patients are summarized in Table 2. We used an RI regimen that was slightly modified from the protocol reported by Barker et al [10] by increasing the TBI dose from 2 to 3 Gy. This modification was made because the patients in our previous RI-CBT pilot study received 2 Gy TBI with Flu and they experienced graft rejection. All patients in the present study experienced pancytopenia after RI-CBT. Although the peripheral blood neutrophil count was less than 500/μL on day 14 in most patients, STR analysis of bone marrow cells on day 14 revealed complete (100%) donor chimerism in 6 of the 12 patients, and 20% to 90% donor chimerism in another 5 patients (Table 2). These results showed unexpectedly early engraftment of donor cells in the bone marrow after the Flu/CY/3 Gy TBI regimen of RI-CBT. The remaining patient (no. 2) experienced graft rejection with the recovery of autologous hematopoiesis. With the exception of 1 patient who was HLA matched, the actual HLA disparity in the other 11 patients was found by genetic typing to be 2 to 4 alleles. Eleven patients showed chimerism in the bone marrow at day 14, and 10 of these patients achieved neutrophil recovery (>500/μL) at a median of 17 days (range, 11-24 days). Platelet recovery was achieved at a median of 32 days (range, 26-44 days) in 5 of the 10 patients who showed neutrophil recovery (Table 2).

3.4. Event-Free and Overall Survival

With a median follow-up of 13 months, the median overall survival time of the entire cohort was 493 days (range, 426-565 days). Overall survival and disease-free survival rates of these 12 patients were 41.7% and 33.3%, respectively (Figure 1).

3.5. Graft-versus-Host Disease

The cumulative incidence of acute GVHD of grade II to IV in these patients conditioned with the Flu/CY/3 Gy TBI regimen was 62.5%. Thirty-three percent of the patients developed chronic GVHD (Table 2).

Table 2.
Outcome of Patients after Reduced-Intensity Conditioning and Cord Blood Transplantation*

Patient No.	Donor Engraftment in Bone Marrow, % Day 14	Day 35	Day 100	Time to Neutrophils >0.5 × 10^9/L, d	Time to Platelets >20 × 10^9/L, d	Acute GVHD	Chronic GVHD	Current Status	Cause of Death
1	17	100	100	24	44	I	No	Dead, d 412	Disease progression
2	0	0	0	Never	Never	NE	NE	Alive, d 565+	
3	72	100	100	20	32	I	Limited	Alive, d 564+	
4	53	100	100	11	37	II	No	Alive, d 493+	
5	100	NE	NE	20	Never	NE	NE	Dead, d 23	Encephalopathy
6	100	100	NE	23	Never	III	NE	Dead, d 40	TMA
7	84	100	100	14	26	I	No	Alive, d 472+	
8	100	100	NE	16	Never	III	NE	Dead, d 71	TMA
9	100	100	NE	17	Never	II	No	Dead, d 117	GI bleeding
10	100	100	100	13	27	II	Limited	Alive, d 426+	
11	100	100	NE	17	Never	NE	NE	Dead, d 44	TMA
12	95	100	NE	Never	Never	NE	NE	Dead, d 37	TMA

*GVHD indicates graft-versus-host disease; Never, neutrophil count never becomes >0.5 × 10^9/L, or platelet count never becomes >20 × 10^9/L; NE, not evaluable; TMA, thrombotic microangiopathy; GI, gastrointestinal.

3.6. RRT and TRM

No patient developed obvious RRT as defined by the criteria reported by Bearman et al [3]. However, the TRM rate before day 100 in patients conditioned with this Flu/CY/3 Gy TBI regimen was 41.7% (5 of 12 patients). Causes of death were central nervous system complications (encephalopathy) (n = 1) [21] and thrombotic microangiopathy (n = 4).

4. Discussion

An essential mechanism of the efficacy of SCT is related to the GVT effect, in which donor T-cells eradicate tumor cells [1,2]. RI preparative regimens for SCT aim to reduce RRT and enhance GVT effects in the treatment of hematologic malignancies [4,5]. The use of CB has markedly expanded the application of SCT to include patients without HLA-matched donors [7,8]. In particular, SCT using an RI-CBT protocol should be applicable to a number of patients not eligible for conventional HLA-matched myeloablative SCT [9]. However, the optimal regimen for RI-CBT has not yet been firmly established.

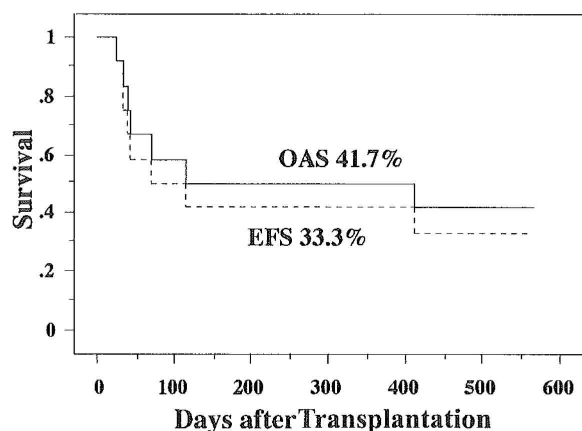

Figure 1. Kaplan-Meier estimates of overall survival (OAS) and event-free survival (EFS).

A major concern regarding CBT with RI conditioning for adult patients is graft failure. Barker et al [10] reported that when 43 adult patients with high-risk or advanced hematologic malignancies were given single- or double-unit CB infusions after conditioning with 2 different Flu-based regimens, engraftment was established in 30 patients with a low incidence of acute GVHD, despite the use of grafts mismatched at 1 or 2 HLA alleles, and resulted in 1-year disease-free survival rates of 21% to 41%. Using the slightly modified Flu/CY/3 Gy TBI regimen, we obtained a comparable survival rate (Figure 1), even though we used only a single unit of CB.

In conventional CBT, no more than 2 HLA mismatches at serologically defined class I HLA-A and HLA-B alleles and DNA-typed class II HLA-DRB1 alleles are considered the criterion for CB graft selection [22-24]. In RI-CBT, it is not clear how many HLA mismatches are acceptable, but most clinicians use CB mismatched at not more than 2 HLA alleles [10-12]. In our CB selection, all of the HLA disparity was at the level of 0 to 2 mismatches according to serologic HLA class I and class II typing. However, high-resolution DNA typing of class I HLA-A and HLA-B and class II DRB1 revealed that the HLA disparity was in fact 2 to 4 mismatches in most patients (Table 1). Thus, genetic HLA disparities were higher than those detected by serologic typing. It is clear that in unrelated CBT, high-resolution DNA typing reveals greater genetic disparity. The effect of this greater HLA genetic disparity on transplantation outcome should be analyzed in a future study.

Engraftment of donor cells is usually defined by neutrophil recovery in the peripheral blood. Early evaluation in the bone marrow before neutrophil recovery in the peripheral blood has not been described. In the present study, an analysis of bone marrow chimerism at day 14 showed unexpectedly early engraftment that had been previously unrecognized in RI-CBT. Early marrow engraftment was paralleled by neutrophil recovery in most patients, suggesting that day 14 bone marrow chimerism does predict donor engraft-

ment. The one exception in our study was a patient (no. 12) with severe infection in whom neutrophil recovery was aborted after early marrow engraftment (Table 1). Our results suggest that CB with 2 to 4 HLA mismatches is able to engraft in bone marrow as early as day 14 following preconditioning with the Flu/CY/3 Gy TBI regimen.

Overall survival and disease-free survival rates were 41.7% and 33.3%, respectively, in our patients (Figure 1). As is shown in Table 1, our patients were in markedly advanced stages of their hematologic malignancies. Ten of these patients had refractory disease, and 2 were in advanced complete remission after having relapsed following previous allogeneic SCT. Such patients have an extremely poor prognosis with conventional salvage therapy. Despite the low-intensity preparative regimen, the therapeutic responses obtained in the present study suggest that RI-CBT exerts antitumor activity through GVT effects. Overall survival will likely improve once the optimal conditions for RI-CBT in terms of types of hematologic malignancy, disease stage, and conditioning regimen are fully established.

The incidence of acute GVHD of grade II to IV in our patients was 62.5%, which is higher than the 44% incidence reported by Barker et al [10]. This high GVHD rate may be related to our early tapering of GVHD prophylaxis. Because most of our patients had refractory hematologic disease, we intended to eradicate the malignancy through the GVT effect by an early induction of GVHD [18]. The appearance of GVHD due to early tapering of GVHD prophylaxis seemed to be related to the occurrence of thrombotic microangiopathy (TMA). In our patients, the TRM rate within 100 days was 41% (Table 2). This rate is identical to that reported recently for RI-CBT in the treatment of malignant lymphoma [25]. The major causes of TRM in our patients were TMA and encephalopathy (Table 2). Kishi et al showed that central nervous system complications such as encephalopathy are fatal complications after RI-CBT [21]. We think that early tapering of GVHD prophylaxis induces acute GVHD and that this induction may be related to the subsequent appearance of TMA or encephalopathy. Furthermore, infection early after RI-CBT also seemed to be related the appearance of TMA. Additional strategies to enhance the GVT effect and reduce GVHD and TRM should therefore be developed.

Our study indicated that RI-CBT using Flu/CY/3 Gy TBI preconditioning allows early bone marrow engraftment and, despite the fact that our patient cohort was small, demonstrated the feasibility of RI-CBT for adult patients with hematologic malignancy. Our RI-CBT protocol is currently associated with a high TRM rate; therefore, further studies are needed to optimize this therapy to minimize the adverse effects and maximize GVT effects.

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Brief report

Allogeneic stem-cell transplantation with reduced conditioning intensity as a novel immunotherapy and antiviral therapy for adult T-cell leukemia/lymphoma

Jun Okamura, Atae Utsunomiya, Ryuji Tanosaki, Naokuni Uike, Shunro Sonoda, Mari Kannagi, Masao Tomonaga, Mine Harada, Nobuhiro Kimura, Masato Masuda, Fumio Kawano, Yuji Yufu, Hiroyoshi Hattori, Hiroshi Kikuchi, and Yoshio Saburi

Sixteen patients with adult T-cell leukemia/lymphoma (ATL) who were all over 50 years of age underwent allogeneic stem cell transplantation with reduced-conditioning intensity (RIST) from HLA-matched sibling donors after a conditioning regimen consisting of fludarabine (180 mg/m²), busulfan (8 mg/kg), and rabbit antithymocyte globulin (5 mg/kg). The observed regimen-related toxicities and nonhematologic toxicities were all found to be acceptable. Disease relapse was the main cause of treatment failure. Three patients who had a relapse subsequently responded to a rapid discontinuation of the immunosuppressive agent and thereafter achieved another remission. After RIST, the human T-cell leukemia virus type 1 (HTLV-1) proviral load became undetectable in 8 patients. RIST is thus considered to be a feasible treatment for ATL. Our data also suggest the presence of a possible graft-versus-ATL effect; an anti-HTLV-1 activity was also found to be associated with this procedure. (Blood. 2005;105:4143-4145)

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Introduction

Therapeutic trials to improve the dismal prognosis of adult T-cell leukemia/lymphoma (ATL) among elderly persons who are infected with human T-lymphotropic virus type 1 (HTLV-1) have so far been unsuccessful.[1-5] However, there have been a few encouraging reports on allogeneic stem cell transplantation (alloSCT) for selected populations of patients with ATL.[6-9] Although most of the patients who were treated successfully in these studies received grafts from HLA-identical siblings and the patients were younger than the average age for patients with ATL, the main cause of treatment failure after alloSCT remains transplant-related complications such as acute graft-versus-host disease (aGVHD). Recent advances have now allowed alloSCT to be extended to older patients through the use of reduced-intensity conditioning regimens.[10-12] We therefore conducted a phase 1 clinical trial of alloSCT with reduced-conditioning intensity (RIST) to clarify whether this newly developed procedure is feasible for ATL patients over 50 years of age.

Study design

The eligible patients ranged from 50 to 70 years of age and met the diagnostic criteria for ATL.[13] The patients were required to be in either complete remission (CR) or partial remission (PR) at the time of registration[5] and to have an HLA-identical sibling donor. All patients and donors gave their written informed consent to participate in this study, which was approved by the institutional review board of each participating institution.

The conditioning regimen consisted of fludarabine (180 mg/m²), busulfan (8 mg/kg), and rabbit antithymocyte globulin (ATG; 5 mg/kg) as reported.[10] Granulocyte colony-stimulating factor-mobilized peripheral blood (PB) grafts from the donors were transplanted. To prevent GVHD, cyclosporine (CsA) was administered intravenously (3 mg/kg/d). The severity of GVHD was graded according to the consensus criteria.[14] The degrees of donor-recipient chimerism and HTLV-1 proviral DNA in PB mononuclear cells (MNCs) were quantified according to published methods.[15,16] The primary end points of this study were either engraftment, as judged by the achievement of complete donor chimerism before day 90, or the occurrence of early transplant-related mortality (TRM) before day 100 after RIST. We therefore registered 16 patients according to the Simon 2-step design.[17] The overall survival (OS) and event-free survival (EFS) were estimated by the Kaplan-Meier method. The log-rank test was used to compare the OS and EFS between the subgroups.

Results and discussion

Clinical results

The median ages of the patients and donors were 57 and 54 years, respectively. Because one patient (UPN11) received extra medication during the conditioning phase due to rapid disease progression, the patient was considered as evaluable only for engraftment. One

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Table 1. Patient characteristics and outcomes

UPN	Age, y/sex	ATL subtype	Donor HTLV-1 antibody	Complete chimerism, PB MNCs, > 90% of donor cells, d	GVHD		HTLV-1 proviral load		Outcome	Survival, d
					Acute	Chronic	Before RIST	After RIST, lowest level		
1	67/F	Acute	+	No	0	NE	292	68	LN relapse, d 47, DOD	135
2	61/F	Acute	−	14	IV	No	> 1000	2	LN relapse, d 47, CR after d/c CsA, died of aGVHD	173
3	62/F	Lymphoma	+	28	0	NE	30	43	LN relapse, d 14, DOD	43
4	62/M	Acute	+	14	I	Yes	> 1000	< 0.5	LN and skin relapse, d 28 and CR after d/c CsA	> 1214
5	51/M	Acute	−	42	0	No	709	223	LN and skin relapse, d 21, PR after d/c CsA, DOD	173
6	66/F	Acute	+	14	II	Yes	798	7	CR	> 1177
7	51/M	Acute	−	14	II	Yes	27	< 0.5	CR	> 1162
8	55/F	Lymphoma	+	20	0	No	331	67	LN relapse, d 74, DOD	201
9	53/M	Lymphoma	−	17	II	Yes	236	< 0.5	CR	> 1017
10	54/M	Lymphoma	−	17	II	Yes	440	< 0.5	LN relapse, d 171, CR after chemoradiotherapy	> 910
11	55/M	Acute	+	21	NE	NE	214	NE	NE	NE
12	66/F	Acute	−	14	0	Yes	> 1000	< 0.5	Died of cGVHD and infection	285
13	57/M	Acute	+	15	III	No	> 1000	2	LN and lung relapse, d 182, DOD	266
14	67/F	Lymphoma	−	15	III	No	582	< 0.5	LN relapse, d 62, DOD	219
15	54/M	Acute	+	28	III	NE	> 1000	< 0.5	Died of aGVHD and sepsis	71
16	56/M	Acute	−	14	IV	No	> 1000	< 0.5	Died of aGVHD	126

patient (UPN1) who developed an early relapse failed to achieve complete donor chimerism before day 90 (Table 1). Therefore, 15 of 16 patients were considered to demonstrate successful results for engraftment. Another patient (UPN15) developed early TRM on day 71 after RIST. As previously reported for this regimen, the regimen-related toxicities and hematologic toxicity were all acceptable. No grade 4 nonhematologic toxicity was observed.[10,18,19] Two patients developed fatal grade IV aGVHD while they were not receiving CsA because of an absence of aGVHD and an early disease relapse. Regarding major infectious complications, sepsis in 2 patients, a reactivation of cytomegalovirus in 13, and an Epstein-Barr virus-associated lymphoproliferative disorder in 2 were observed. Of the 12 patients who could be evaluated regarding the response to RIST, 9 exhibited CR at 30 days after RIST. Although the underlying mechanisms are unclear, the CR was considered most likely to be due to the chemotherapeutic effect, the graft-versus-ATL effect, or a combination of both. Disease relapse occurred in 9 patients. Interestingly, 3 patients who had a relapse subsequently achieved a second CR or PR after the rapid discontinuation of CsA. As of December 31, 2004, 5 patients are alive, and 10 had died of either ATL (6) or TRM (4). In all cases, TRM was considered to be related to GVHD (Table 1). The EFS and OS for the 15 patients at 2 years are 20.0% ± 10.3% and 33.3 ± 12.2%, respectively. The OS for patients who did and did not develop aGVHD was 50.0% ± 15.8% and 0%, respectively ($P = .06$).

Kinetics of the HTLV-1 proviral load after RIST

The HTLV-1 proviral load decreased to an undetectable level (< 0.5 copies) within 3 months after RIST in 8 patients, specifically, 6 of 8 patients who received grafts from HTLV-1 antibody-negative donors and 2 of 7 patients whose donors were virus carriers (Figure 1). Four of the 5 patients who survived more than 18 months presently continue to demonstrate an undetectable HTLV-1 proviral load. The other long-term survivor whose donor was a carrier (UPN6) showed a high HTLV-1 proviral load without any disease relapse beyond 18 months.

In this first prospective study of RIST for ATL, we clearly demonstrated that RIST from HLA-matched sibling donors is a feasible therapeutic procedure for patients over 50 years of age, as has been reported for other lymphoid malignancies.[20-22] However, the TRM of 27% was not negligible. Notably, 2 of 4 TRMs were related to grade IV aGVHD, and they were induced by a

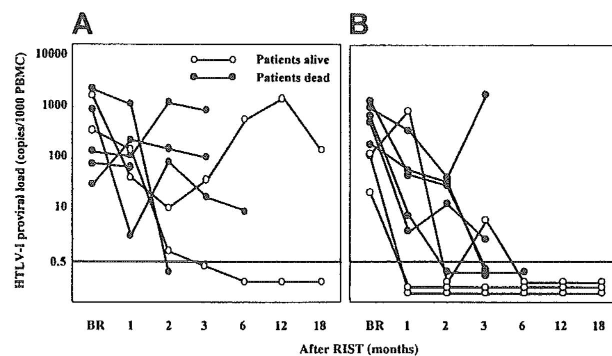

Figure 1. The kinetics of the HTLV-1 proviral load after RIST by different types of donors. Panel B indicates transplants from HTLV-1$^-$ donors; panel A shows results from HTLV-1$^+$ carrier donors. The HTLV-1 proviral load was expressed as copies per 1000 MNCs. A load of less than 0.5 copies/1000 MNCs was considered to be undetectable. ○ indicates patients still alive at end of study; ●, patients that died during study. BR indicates before RIST. The horizontal line at 0.5 indicates detection limit. PBMNC indicates peripheral blood mononuclear cell.

discontinuation of CsA, which indicated the difficulty in the tapering or discontinuation of CsA in RIST. Interestingly, 3 patients who had a relapse responded to a rapid discontinuation of the immunosuppressive agent CsA. Although the difference was not statistically significant, the patients who developed aGVHD tended to show a better OS than those who did not ($P = .06$). These observations thus suggest the presence of a graft-versus-ATL effect in RIST. The dramatic decrease in the HTLV-1 proviral load to an undetectable level after RIST in more than half the patients was unexpected. Similar results, which demonstrated an antiviral effect by SCT for ATL, have been previously described in case reports.[23,24] Two patients who received grafts from HTLV-1$^+$ donors also became negative for viral load after RIST. The uninfected normal donor T cells present in the graft might have overwhelmed the HTLV-1–infected T cells in the unique environment after transplantation. In one patient (UPN6) who received a graft from an HTLV-1$^+$ carrier donor, an increase in the HTLV-1 proviral load without disease relapse was observed beyond 1 year after RIST. The proviral load gradually returned to the donor level after the second year. A temporary proliferation of HTLV-I–infected (nonclonal) donor cells might have occurred due to some unknown etiology.

We have herein shown that RIST is a feasible treatment procedure for ATL patients over 50 years of age. The possible presence of a graft-versus-ATL effect as well as anti–HTLV-1 activity for RIST were also observed. Ganciclovir and prophylactic oral acyclovir were the antiviral agents used in the study. They are effective only for herpes virus and not for retrovirus, and therefore, they possess a negligible anti–HTLV-1 activity. In a separate analysis in this study, Harashima et al found the presence of an HLA class I restricted proliferation of CD8$^+$ cytotoxic T lymphocytes (CTLs), which exhibited a specific reactivity to a certain epitope of the HTLV-1 regulatory protein Tax.[25] These Tax-specific CTLs might therefore play a critical role in eradicating ATL cells in vivo. These results indicate that RIST may be applicable as a new modality for the future treatment for other virus-induced diseases that have a poor prognosis.

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Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma

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Adult T-cell leukemia/lymphoma (ATLL) is a distinct peripheral T-cell neoplasm that is highly resistant to chemotherapy. Several groups, including ours, have reported encouraging results of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for patients with ATLL. To confirm our previous report and to establish the basis for a phase II clinical study, we analyzed 40 allo-HSCT for acute and lymphoma types of ATLL in seven institutions in Japan between 1997 and 2002. All evaluable cases entered complete remission (CR) after allo-HSCT and the median survival time was 9.6 months for all patients. The estimated 3-year overall and relapse-free survival, and disease relapse were 45.3, 33.8 and 39.3%, respectively. Among 10 cases with ATLL relapse, five cases achieved CR again: three by the reduction or cessation of immunosuppressive agents, which suggested a graft-versus-ATLL (GvATLL) effect. However, univariate or multivariate analysis did not show any benefit of graft-versus-host disease (GVHD) on the prevention of relapse. These results suggested that allo-HSCT was effective for some patients with aggressive ATLL, and that the GvATLL effect could be achieved even without GVHD. A new phase II trial to test the efficacy of allo-HSCT for ATLL is warranted.
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Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm associated with the infection of a specific retrovirus, human T-cell lymphotropic virus type-I (HTLV-I).[1–4] ATLL is quite different from other types of non-Hodgkin's lymphoma (NHL): restricted endemic areas including the west coast of Japan, with distinct clinical features such as high frequency of hypercalcemia, a strong predisposition to infection and poor response to chemotherapy.[4,5] Multicenter clinical trials for ATLL conducted by the Japan Clinical Oncology Group (JCOG) have shown that standard-dose chemotherapy for NHL was unable to cure any patients with acute or lymphoma types of ATLL, which is more aggressive than the chronic or smoldering type.[5–7] The best chemotherapy result for those patients was obtained from the phase II trial of a new multidrug regimen containing nine agents with the support of granulocyte colony-stimulating factor (JCOG9303, LSG15 protocol).[8] The overall survival (OS) rate of the 93 patients in this trial was estimated at 31% at 2 years. In these circumstances, high-dose chemotherapy/radiotherapy with hematopoietic stem cell transplantation (HSCT) was applied to those patients, particularly in Japan. The results of allogeneic, not autologous, HSCT reported by several groups, including ours, suggested that it could provide durable clinical responses for some patients with ATLL.[9–14] However, the number of patients in each report was so small that the efficacy of allogeneic HSCT (allo-HSCT) for ATLL is still controversial. In this report, we aimed to extend our previous study to establish the basis for a phase II clinical trial of allo-HSCT for aggressive ATLL by retrospectively collecting a larger number (40) of transplants from seven institutions in Japan. Our results suggested that allo-HSCT is effective for some patients with ATLL, and that there may be a graft-versus-ATLL (GvATLL) effect that cannot be obtained by chemotherapy alone or high-dose therapy with autograft.

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Patients and methods

Study design, data collection and transplantation procedure

This is a retrospective analysis of myeloablative allo-HSCT for patients with ATLL performed at seven institutions in Japan from June 1997 to April 2002. Data on donors and recipients were collected using questionnaires distributed to each participating center in this study. Patients included in this study received intensive conditioning regimens prior to stem cell transplantation. The early results of the four out of 40 cases were reported previously.[14]

Since transplantation was performed following the protocol of each institution, the conditioning regimen or prophylaxis for GVHD varied among institutions (Table 1); however, all but one preconditioning regimens had myeloablative intensity (containing more than 12 Gy of total body irradiation (TBI, 22 cases), 16 mg/kg of busulfan (17 cases) and 140 mg/m^2 of melphalan (one case)).

Definitions of clinical end points and responses

The day of engraftment was defined as the first of 3 consecutive days on which the neutrophil count exceeded 0.5×10^9/l.

Table 1 Patient characteristics and transplant condition

Sex (male/female)	22/18
Median age at transplantation (range)	44 (28–53)
Subtypes of ATLL	
Acute	30
Lymphoma	10
Disease status at transplantation	
CR1	14
CR2	1
PR	13
NC	3
PD	9
Performance status at transplantation	
0	3
1	33
2	3
3	1
Donor	
HLA-matched related	27
HLA-mismatched related	5
HLA-matched unrelated	8
Anti-HTLV-I antibody of donor	
Positive	9
Negatine	27
NE	4
Source of stem cells	
Bone marrow	21
Peripheral blood stem cell	19
Conditioning regimen	
TBI-containing	18
Non-TBI-containing	22
GVHD prophylaxis	
CsA+MTX	28
TCR+MTX	11
TCR	1

NE, not evaluable; CsA, ciclosporin; TCR, tacrolimus; MTX, methotorexate.

Patients who died before day 28 were considered unevaluable for engraftment. Relapse-free survival (RFS) was defined as the time interval from transplantation to the first event (either relapse or death in complete remission (CR)). Acute graft-versus-host disease (aGVHD) was diagnosed and graded at each transplantation center according to the standard criteria with grades 0, I, II, III and IV.[15] Chronic GVHD (cGVHD) was defined according to standard criteria of absent, limited or extensive.[16] One patient, who received transplantation at the stage of progressive disease (PD), died of infection on day 8 after transplantation, and this case was excluded from the analysis of engraftment, response to transplantation and GVHD. The response criteria used in this report followed those established by JCOG.[8] CR was defined as the disappearance of all clinical and radiographic evidence of disease and the normalization of lactate dehydrogenase (LDH). As HTLV-I carriers frequently have a small percentage of abnormal lymphocytes, CR was judged with less than 5% of such cells if the absolute lymphocyte count was less than 4×10^9/l. Partial response (PR) was defined as a $\geqslant$50% reduction of the measurable disease with more than 75% reduction in the absolute abnormal lymphocyte count. LDH had to be decreased to <1.5 of the normal upper limit. PD was defined by $\geqslant$25% increase of the measurable disease or the appearance of new lesions while under treatment. No change (NC) was defined by an intermediate response between PR and PD.

Statistical analysis

The Kaplan–Meier method was used to estimate OS and RFS. The 95% confidence intervals of 3-year OS and RFS were calculated. For analyzing the relapse after transplantation, the cumulative distribution function of relapse was calculated. The differences in the OS and RFS rates between the groups of transplantation-related factors were compared by the log-rank test. Among those who reached CR after transplantation, the differences in the proportion of ATLL relapse between the groups of transplantation-related factors were compared by the χ^2 test. Furthermore, simultaneous effects of prognostic factors on survival (OS and RFS) and relapse were analyzed using multivariate regression analysis based on the Cox's proportional hazards model and the linear logistic model, respectively. The most appropriate models were selected based on Akaike's information criteria (AIC). All analyses were performed using SAS (Statistical Analysis System Inc., Cary, NC, USA) software.[17]

Results

Patient characteristics and transplant conditions

Data on 40 transplantations were collected. The clinical characteristics of patients are summarized in Table 1. All patients received standard-dose chemotherapy before the procedure of transplantation, and 28 patients (70%) showed a clinical response to the previous chemotherapy. Nine related donors showed a positive result for the anti-HTLV-I antibody. The peripheral blood mononuclear cells of these donors were subjected to Southern blot analysis to examine the monoclonal integration of HTLV-I provirus into the genome, and all nine donors were confirmed as carriers of HTLV-I.

Engraftment and response to transplantation

The median number of cells transplanted was 3.10. (1.20–9.20) $\times 10^8$/kg of nucleated cells for bone marrow transplantation (21 cases) and 3.94 (1.20–8.30) $\times 10^6$/kg of CD34-positive cells for peripheral blood stem cell transplantation (19 cases). All evaluable patients (39 recipients) achieved engraftment and the median time of neutrophil recovery to a level more than 0.5×10^9/l was 15 days (range 11–36). No case suffered from late graft rejection. All patients who received transplantation during PR disease status (13 cases) achieved CR after transplantation. Of 11 patients with resistant disease (three NC and eight PD) at the time of transplantation, 10 patients (except one PD case) achieved CR.

GVHD and transplantation-related early toxicity

aGVHD developed in 26 of 39 patients (66.7%), and 15 out of 31 patients developed cGVHD (Table 2). There were 21 deaths after transplantation, and 16 were related to adverse events of transplantation (Table 2). Within 6 months after transplantation, 15 patients died: 13 were lost to adverse events of transplantation.

Table 2 Transplantation outcome

Alive/dead	19/21
Cause of death	
<6 months after transplanatation	
GVHD	6
Infection	3
TMA	3
Hepatitis	1
Disease progression	2
⩾6 months after transplantation	
Infection	2
Disease progression	2
Bronchiolitis obliterance	1
Unknown[a]	1
Relapse of ATLL	10
Acute GVHD	
Grade 0	13
Grade I	8
Grade II	11
Grade III	4
Grade IV	3
Chronic GVHD	
Limited	4
Extensive	11

[a]Sudden death.
TMA, thrombotic microangiopathy.

Survival and relapse of ATLL

The median survival time of all cases after transplantation was 9.6 months (range 0.3–63.6 months), and the median observation time of 19 surviving patients was 22.7 months (3.7–63.6) (Table 2 and Figure 1). Estimated OS and RFS rates at 3 years were 45.3% (95% confidence interval (CI): 31.8–58.8%) and 33.8% (95% CI: 17.2–49.4%), respectively (Figures 1 and 2). ATLL relapse occurred in 10 patients after transplantation (Table 2). The estimated risk of disease relapse at 3 years was 39.3% (95% CI: 19.7–58.9%) (Figure 3). However, five achieved another CR: three by reduction or cessation of immunosuppressive agents, one by standard-dose chemotherapy and one by local radiation therapy. Among these five patients, three maintained CR over 1 year.

Univariate and multivariate analysis for survival and relapse

The univariate analysis for survival identified several pretransplantation and posttransplantation factors (Table 3). The performance status before transplantation had a significant impact on OS and RFS, but the disease status at transplantation did not. The existence of aGVHD or cGVHD did not affect the ATLL relapse, and aGVHD (grades I–IV) had a significant negative impact on OS and RFS. Patients with grade 0 or I aGVHD showed no significant difference on their OS or RFS from those with stronger aGVHD. Transplantation from a carrier donor was associated with better survival than from an HTLV-I-negative donor. Multivariate analysis selected several factors for survival and relapse (Table 4). Among factors selected, the presence of aGVHD had a significant negative impact on both OS and RFS. For RFS, transplant from related donor showed better results. Transplantation from a carrier donor had

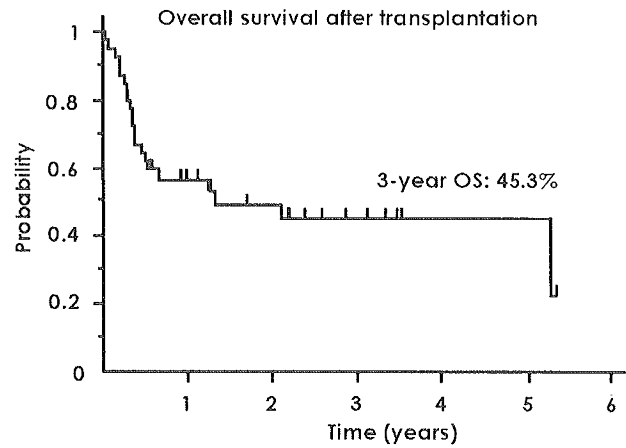

Figure 1 OS after transplantation. The estimated 3-year OS rate was 45.3%.

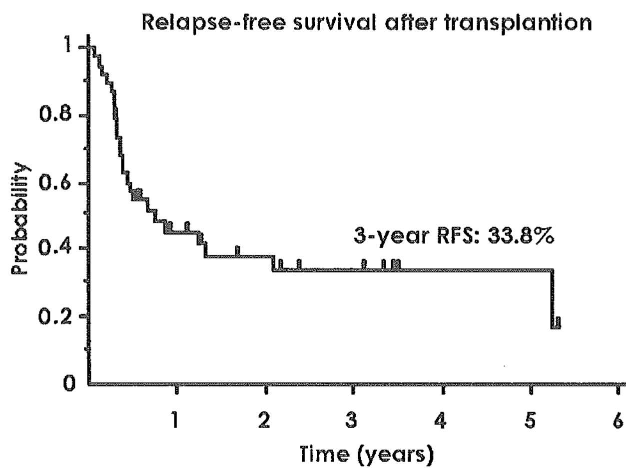

Figure 2 RFS after transplantation. The estimated 3-year RFS rate was 33.8%.

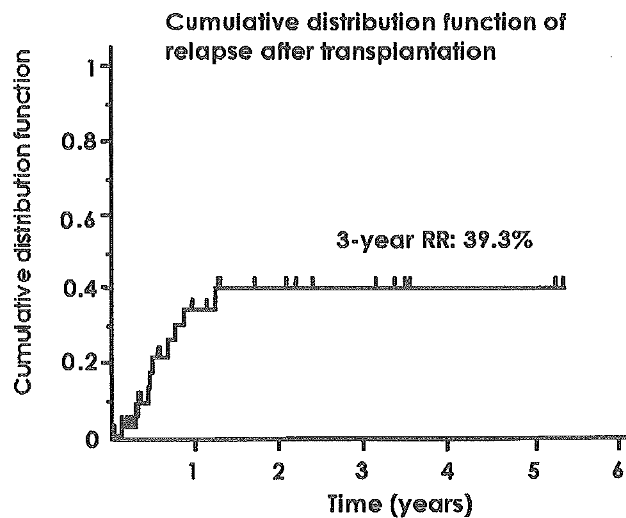

Figure 3 Cumulative distribution function of relapse after transplantation.

Table 3 Univariate analysis for OS, RFS and relapse

	No. of patients (for OS and RFS)	OS (median survival, days)		RFS (median survival, days)		No. of patients (for Relapse)	No. of relapse	
At diagnosis								
Subtype of ATLL								
Acute	30	208	$P=0.6762$	208	$P=0.7613$	29	6	$P=0.1574$
Lymphoma	10	405		174		9	4	
Transplantation-related factors								
Time from diagnosis to transplantation								
<220 days	20	192	$P=0.8344$	168	$P=0.6371$			
⩾220 days	20	405		247				
Age								
<43 years	15	216	$P=0.5148$	216	$P=0.2198$			
⩾43 years	25	340		134				
PS								
0	3	615	$P<0.0001$	308	$P<0.0001$			
1	33	362		201				
2	3	122		122				
3	1	8		NE				
Conditioning regimen								
TBI-based	22	405	$P=0.9234$	216	$P=0.4448$	20	6	$P=0.6587$
Busulfan-based	17	237		188		17	4	
Status 1								
CR1 or CR2	16	216	$P=0.6548$	262	$P=0.9745$	16	4	$P=0.8752$
Others	24	463		161		22	6	
Status 2								
CR1, CR2 or PR	29	406	$P=0.1586$	224	$P=0.4110$	29	9	$P=0.2357$
Others	11	135		128		9	1	
aGVHD1								
O	13	463	$P=0.0315$	340	$P=0.0100$	13	3	$P=0.7437$
Others	26	151		128		25	7	
aGVHD2								
0 or I	21	449	$P=0.1087$	262	$P=0.0918$	21	6	$P=0.7256$
Others	18	151		135		17	4	
cGVHD								
(+)	15	456	$P=0.9157$	372	$P=0.5849$	15	6	$P=0.1927$
(−)	16	450		216		16	3	
Relapse								
(+)	10	405	$P=0.6718$					
(−)	29	278						
Donor source								
Related	32	351	$P=0.1308$	208	$P=0.0227$	32	6	$P=0.0144$
Unrelated	8	168		139		6	4	
Donor HTLV-I								
(+)	9	463	$P=0.0462$	436	$P=0.1257$	9	4	$P=0.2484$
(−)	27	171		123		25	6	

significantly higher incidence of relapse than that from a noncarrier donor.

Discussion

This retrospective study reported the outcome of allo-HSCT for 40 patients with ATLL who received intensive treatment (myeloablative intensity in most cases) as a conditioning regimen before transplantation. The OS and RFS rates at 3 years were 45.3 and 33.8%, respectively, and these results were comparable with our previous report of 10 cases.[14] Patients in this study were diagnosed with either the acute or lymphoma type of ATLL, which usually shows an aggressive clinical course.[7] Among 11 evaluable patients who received transplantation at the point of resistant disease (NC and PD), all but one