factor and PAI-1 [11-13,15-17]. Our data presented here also support these previous reports.

In conclusion, rTM could significantly reduce HMGB1 and PAI-1 levels after HSCT. Additionally, patients who received preventive rTM exhibited significantly lower frequencies of VOD and/or TMA, as well as aGVHD compared to patients who did not receive rTM. Therefore, the results of our multi-institutional study suggest that trTM is beneficial when used as a preventive therapy for established TAC after HSCT.

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Case Report

Primary follicular lymphoma of the spleen incidentally found in a patient with alcohol- and hepatitis C-related liver cirrhosis

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Abstract: Primary splenic lymphoma is rare as non-Hodgkin lymphomas. Splenic infiltration of lymphoma cells may cause splenomegaly in many cases. However, splenomegaly is caused not only by tumor involvement but also by non-tumorous disorders. One of the most prevalent non-neoplastic causes is portal hypertension mostly due to liver cirrhosis. On the other hand, liver cirrhosis may underlie various extrahepatic manifestations including development of B-cell non-Hodgkin lymphomas. Here, we report a case of primary follicular lymphoma of the spleen in a patient with liver cirrhosis related to hepatitis C and alcohol. The lymphoma was incidentally found in an enlarged spleen resected palliatively to alleviate symptomatic pancytopenia of the patient. The main characteristic of our case is an incidental finding of a rare situation brought by careful pathological examination. Our case illustrates the importance to recognize a possibility of co-occurrence of chronic liver disease and extrahepatic lymphoma.

Keywords: Follicular lymphoma, spleen, alcohol, hepatitis C, liver cirrhosis

Introduction

Primary splenic lymphoma is rare, occurring in no more than 1% of cases of non-Hodgkin lymphoma [1]. Among primary splenic lymphoma in Japanese patients, follicular lymphoma (FL) comprises 5.98% of them [2].

FL is a subtype of low grade B-cell lymphoma [3]. Its postulated normal counterparts are centrocytes inside germinal centers (GC) of secondary lymphoid follicles in lymph nodes. FL consists of follicle-like structures composed of centrocyte-like tumor cells expressing CD10 and BCL2 protein. Expression of BCL2 proteins in FLs is a consequence of t(14;18)(q32;q21) chromosomal translocation, in which BCL2 locus is under the control of IGH promoter. FL manifests itself not only as nodal but also as extranodal lesions including splenic one. Pure extranodal presentations are uncommon (9% in one survey) [4].

Splenic infiltration of lymphoma cells may cause splenomegaly in many cases. However, splenomegaly is caused not only by primary or secondary involvement of tumors, but also by non-tumorous disorders. One of the prevalent causes for splenomegaly is portal hypertension associated with liver cirrhosis or other hepatic disorders. Splenomagaly may cause symptomatic pancytopenia, in which case palliative splenectomy may be chosen. On the other hand, liver cirrhosis may underlie various extrahepatic manifestations including development of B-cell non-Hodgkin lymphomas.

Here, we report a case of primary follicular lymphoma of the spleen in a patient with liver cirrhosis related to hepatitis C and alcohol. The lymphoma was incidentally found in an enlarged spleen resected palliatively to alleviate symptomatic pancytopenia of the patient.

Case report

A 56-year-old Japanese female was admitted to our hospital for splenectomy. She had been suffering from liver cirrhosis related to alcohol and infection with hepatitis C virus (HCV) (**Figure 1**).

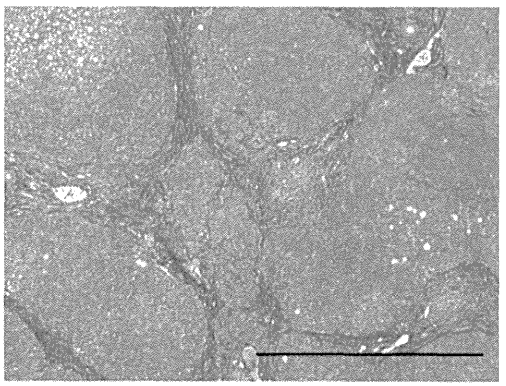

Figure 1. Histological image of the liver cirrhosis. Regenerative nodules are completely encircled by bridging fibrosis, which is stained blue with Azan Mallory method. Original magnification: × 40, Bar: 1 mm.

Physical examination and abdominal computerized tomography (CT) with contrast enhancement revealed cirrhotic liver and enlarged spleen (around 17 × 8 cm in size) with vague nodularity (**Figure 2A**). Peripheral blood examination showed pancytopenia with 39.7 × 10^2/μL of white blood cell count (normal range: 40-90), 10.3 g/dL of hemoglobin (normal range: 11.5-15), and 6.7 × 10^4/μL of platelet count (normal range: 15-35). Her pancytopenia was considered to be attributed to hypersplenism caused by portal hypertension due to liver cirrhosis. Palliative splenectomy was chosen to relieve her symptomatic pancytopenia.

Pathological examination of the resected spleen revealed apparent increase in number of lymphoid follicles (white pulps). At low power magnification, multiple follicle-like structures were observed (**Figure 2B**). The density of the follicle-like structrures appeared to be increased compared with follicles observed in the normal spleen. This was not typical as the spleen of cirrhotic patients, where lymphoid follicles in the white pulp are often atrophied due to chronic congestion. Careful examination revealed slightly ambiguous polarity in GC-like structures in these follicle-like lesions (**Figure 2C**). That is, centrocyte-like cells in the follicle-like lesions were homogeneous in morphology and size, compared with centrocytes observed in the normal GCs (**Figure 2D**). Moreover, tingible body macrophages were not apparent in the GC-like structures (**Figure 2C**).

Immunohistochemistry revealed that centrocyte-like cells in the GC-like structures with the unclear polarity were stained positive for CD20 (**Figure 3A**), CD79a (data not shown), CD10 (**Figure 3C**), and BCL2 (**Figure 3D**), while they were negative for CD3 (**Figure 3B**), CD23 (data not shown), and Cyclin D1 (data not shown). Centroblast-like cells were not apparent. Preservation of normal reactive GCs was not evident. Diagnosis of FL, grade 1, in the spleen was made. ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET)/CT examination after the splenectomy revealed several para-aortic lymph nodes up to 8 mm in size, but no significant uptake of FDG was detected (data not shown). No other hotspots of FDG uptake were detected, including the bone marrow (data not shown). The patient was carefully followed up without chemotherapy. During 2-year follow-up by FDG-PET/CT after the diagnosis, there have been no significant changes in size of these lymph nodes without significant uptake of FDG. These results confirmed that the final diagnosis of the splenic lesion was primary FL of the spleen at clinical stage I.

After the diagnosis of follicular lymphoma, interferon therapy for the liver cirrhosis was initiated. During the course of the therapy, multiple space-occupying lesions were pointed out in the liver by gadoxetic acid-enhanced magnetic resonance imaging. They were diagnosed as hepatocellular carcinomas by needle biopsy (data not shown), and were treated by radiofrequency ablation and/or transcatheter arterial chemoembolization.

Discussion

In this paper, we reported a case of primary FL of the spleen incidentally found in a patient with alcohol- and HCV-related liver cirrhosis. Mollejo M *et al.* reported clinicopathological characteristics of 32 cases of primary splenic FL [5]. They described that splenic FL consists of 2 subgroups with different clinicopathological characteristics: (a) classical FL with t(14; 18) and CD10 expression, usually diagnosed at advanced stages, and (b) BCL2-negative FL cases, with higher histologic grade and more frequently initially seen as disease restricted to the spleen [5]. The lymphoma in our case is histopathologically designated as classical FL, but is considered to be localized to the spleen. The cause of the discrepancy may be related to a particular clinical background of this patient, that is, alcohol- and HCV-related liver cirrhosis, which was not described by Mollejo M *et al.* [5].

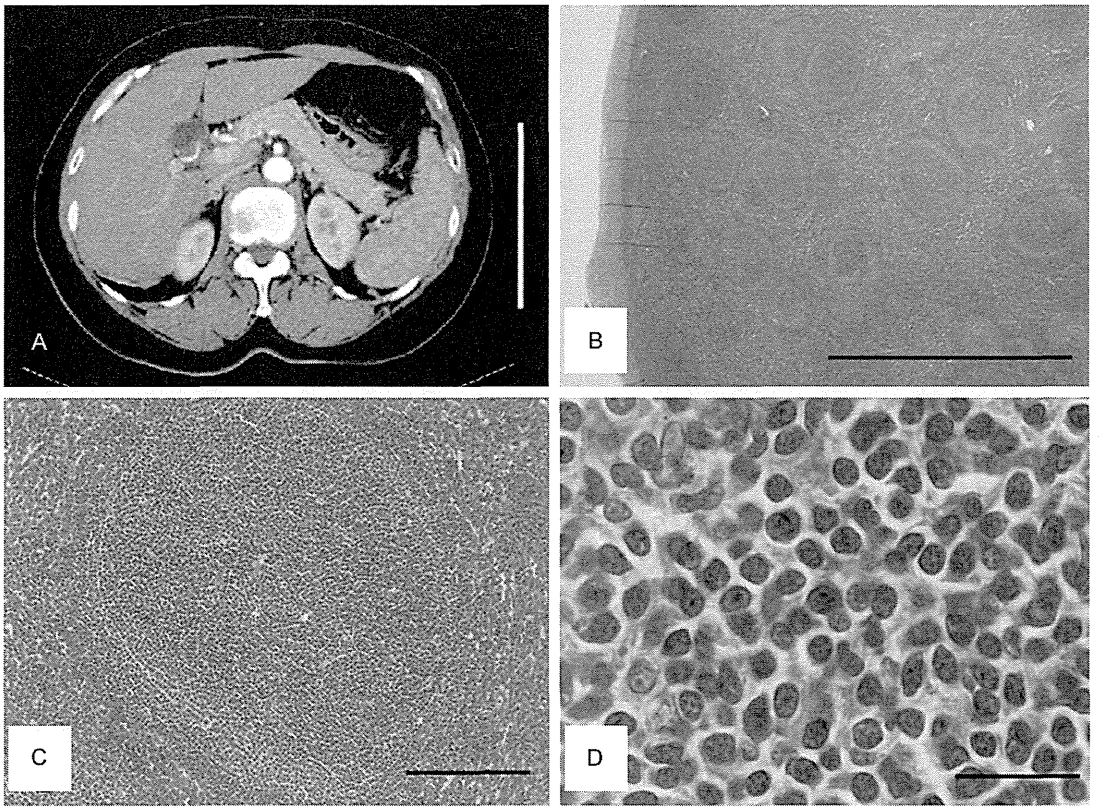

Figure 2. Radiological and histological views of the spleen. A. Abdominal CT with contrast enhancement. An enlarged spleen is shown in the right half of the figure. Bar: 20 cm. B-D. Representative HE images of the resected spleen. B. At low power magnification, multiple follicle-like structures are observed. Original magnification: × 20, Bar: 2 mm. C. At high power magnification, the follicle-like structure is composed of homogeneous proliferation of small lymphoid cells. Polarization observed in the normal germinal center (GC) appears unclear. Original magnification: × 100, Bar: 200 μm. D. Tumor cells are centrocytes-like in normal GCs. Apparently no centroblast-like cells are observed. Original magnification: × 1000, Bar: 20 μm.

It is generally known that liver cirrhosis is not only a risk factor for hepatocellular carcinoma but also associated with a variety of extrahepatic abnormalities. There have been several retrospective cohort studies on malignancies associated with liver cirrhosis [6, 7]. According to one study, the frequency of lymphomas was ranked as the third, following lung and breast cancers [6]. In the case of HCV-related liver disease, marginal zone lymphoma (in particular splenic marginal zone lymphoma), lymphoplasmacytic lymphoma, diffuse large B-cell lymphoma were reported to be frequently associated [8, 9]. Other than these lymphomas and FL, Mollejo M *et al.* recently described previously underrecognized histological patterns of HCV-associated lymphoproliferative disorders [10]. In addition, there are case reports on primary effusion lymphoma-like lymphomas associated with HCV-related liver cirrhosis [11].

In the case of HCV-associated B-cell lymphomas, three pathological mechanisms have been elaborated for lymphomagenesis [9]. First, continuous stimulation of B cells by viral antigen leads to their consecutive excessive proliferation, just as illustrated by mucosa associated lymphoid tissue (MALT) lymphoma cells stimulated by Helicobacter pylori. Immunological abnormalities may play primary roles in extrahepatic lymphoma development [9]. Second, since HCV is potentially B-lymphotropic [12, 13], HCV replication in B cells may mediate oncogenic effects by intracellular viral proteins, which has been controversial [9]. Third, it has been proposed that HCV infection may induce a high mutation frequency of cellular genes [14], which has also been controversial. Interestingly, one of the most convincing evidences for causal relationships between these lymphomas and HCV is the observation that eradication of HCV

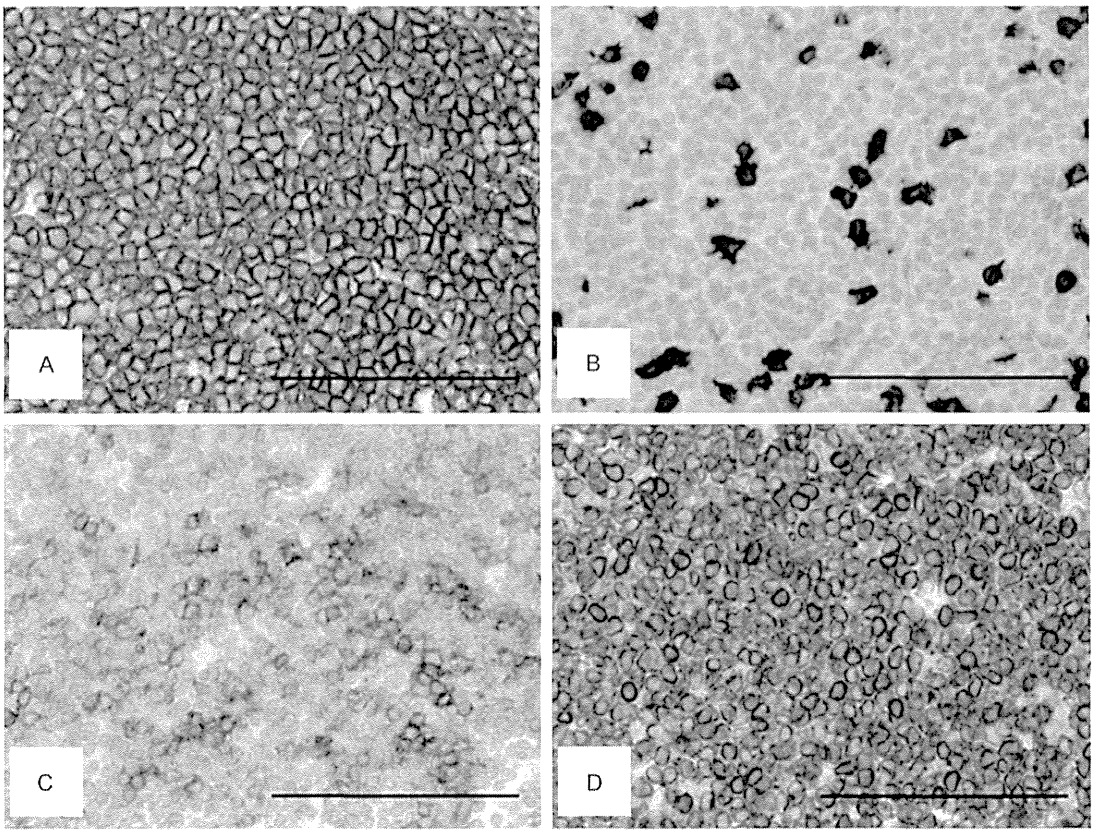

Figure 3. Immunohistochemical analysis of the tumor cells in the spleen. (A) CD20, (B) CD3, (C) CD10, (D) BCL2. Tumor cells are positive for CD20 (A), CD10 (C), and BCL2 (D), but negative for CD3 (B). Original magnification: × 400, Bar: 100 μm.

by antiviral and/or interferon therapy leads to remission of these lymphomas [9, 15]. In our case, no relapse of the lymphoma was apparently observed during interferon therapy against HCV-related liver cirrhosis. It is tempting to speculate that the FL in our case responded to the interferon therapy. On the other hand, in anthracycline-based chemotherapy coupled with rituximab for B-cell non-Hodgkin lymphomas, care should be taken not only to reactivation of clinically silent HCV but also to hepatotoxicity of the drugs [9].

In conclusion, to our knowledge, our paper is the first report on primary FL of the spleen incidentally found in splenectomy specimen of a patient with alcohol- and hepatitis C-related liver cirrhosis. The low-power view of the spleen mimicked reactive lymphoid hyperplasia; however this was not typical as the spleen of cirrhotic patients, where lymphoid follicles in the white pulp are often atrophied due to chronic congestion. Careful examination led to the correct diagnosis of FL of the spleen. One of the lessons from this case will be that it is clinically important to recognize a possibility of co-occurrence of chronic liver disease and extrahepatic lymphoma. Close collaboration between hepatologists and hematologists will be desired for appropriate management of patients with liver cirrhosis.

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Disclosure of conflict of interest

None.

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ORIGINAL ARTICLE

Etoposide-containing conditioning regimen reduces the occurrence of hemophagocytic lymphohistiocytosis after SCT

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Hemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening disease of severe hyperinflammation caused by uncontrolled proliferation of activated lymphocytes and macrophages that secrete high amounts of inflammatory cytokines. HLH occurring after SCT is difficult to diagnose. It is characterized by severe clinical manifestations and high mortality. Despite current therapeutic approaches, outcomes remain poor. We analyzed the incidence and risk factors of HLH after SCT and the response to treatment and prognosis of 554 patients with HLH after SCT. The cumulative incidence of HLH after SCT was 4.3% (24/554). Use of etoposide in the conditioning regimen was only factor that reduced HLH after SCT ($P = 0.027$). All patients who received autologous transplantation were successfully treated. Patients with liver dysfunction (for example, high total bilirubin level, prolonged prothrombin time and high level of fibrinogen degradation products) had a poor response to treatment for HLH. Physicians should be cautious of HLH, while not using etoposide for conditioning regimen.

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Keywords: hemophagocytic lymphohistiocytosis; transplantation; etoposide

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening disease of severe hyperinflammation caused by uncontrolled proliferation of activated lymphocytes and macrophages that secrete high amounts of inflammatory cytokines.[1] Although HLH is rare in adults, this disease is sometimes observed in children. HLH is usually fatal without treatment. Typical clinical findings include hepatosplenomegaly and prolonged fever that is usually unresponsive to antibiotic therapy. Lymphadenopathy, different kinds of rash, edema and jaundice are less frequent manifestations. Laboratory findings include cytopenias (usually beginning with thrombocytopenia and evolving into severe pancytopenia), hyperferritinemia, elevated transaminases, hypofibrinogenemia, hypertriglyceridemia, hypoalbuminemia and hyponatremia.[1] Additional immunological findings include elevated soluble IL-2 receptor and reduced Natural Killer cell cytotoxicity. Many patients with HLH show signs of disseminated intravascular coagulation. Hemophagocytosis can be absent in the early stages of the disease,[2,3] but serial BM aspirations may reveal hemophagocytosis later in the course. It is important to note that the diagnosis of HLH does not depend on this morphological finding. HLH occurring after SCT is particularly difficult to diagnose. It is characterized by severe clinical manifestations and high mortality.[4–7] Despite current therapeutic approaches, outcomes remain poor. The present study analyzed HLH after SCT in patients in Hokkaido prefecture in Japan.

PATIENTS AND METHODS

Patients

A total of 554 patients (310 males, 244 females) with different hematological malignancies, solid tumor, metabolic abnormalities and immunodeficiencies underwent SCT between January 2007 and December 2011 at one of 11 institutes in Hokkaido prefecture. The ages of the patients at transplantation ranged from 0 to 73 years (median, 49 years). In all, 167 patients had non-Hodgkin's lymphoma, 119 had AML and 76 had ALL. In all, 192 patients had other diseases, including 61 with multiple myeloma, 37 with myelodysplastic syndrome, 20 with solid tumors, 13 with aplastic anemia (AA), 13 with Hodgkin's lymphoma, 11 with CML, 10 with adult T-cell leukemia and 27 with other diseases, including metabolic diseases and immunodeficiency.

Treatment protocols

The origin of the stem cells was as follows: 219 patients received BMT, 232 patients underwent PBSC transplantation and 103 patients received cord blood SCT (CBT). The transplantation donors were related donors in 126 cases and unrelated donors in 240 cases, and autologous transplantation was performed in 188 cases. The conditioning regimen contained BU for 149 patients, etoposide for 183 patients, fludarabine for 208 patients, melphalan for 151 patients, CY for 288 patients and anti-thymocyte globulin for 10 patients. In addition, TBI was used as part of the conditioning regimen for 309 patients. Reduced intensity conditioning SCT was used for 242 patients, and myeloablative conditioning SCT was selected for 312 patients. Prophylaxis for GVHD was performed with cyclosporine for 147 patients, tacrolimus for 208 patients, MTX for 294 patients, methylprednisolone for 22 patients and mycophenolate mofetil

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for 9 patients. Data were analyzed as of 1 July 2012. Median follow-up time was 12.9 months from SCT. The analysis protocol of the present study was approved by the Institutional Review Board of Sapporo Hokuyu Hospital.

Definition of HLH

We used the criteria for HLH after SCT that were described by Takagi *et al.*[7] A diagnosis of SCT-HLH required both major criteria, or one major and all four minor criteria. The first major criterion was engraftment failure, delayed engraftment or secondary engraftment failure after SCT, and the second major criterion was histopathological evidence of hemophagocytosis. The four minor criteria were high-grade fever, hepatosplenomegaly, elevated ferritin and elevated serum lactate dehydrogenase. Although progressive cytopenia has formed the backbone of the previous criteria, this criterion was not used in this study, considering the post-SCT setting.

Statistical analysis

A Mann–Whitney *U*-test or χ^2 test was used to compare patients who did versus those who did not develop HLH. Analysis of survival was performed using the Kaplan–Meier method, with differences compared by log-rank test. Cox proportional hazards regression analysis was used for analysis of risk factor of HLH. Statistical analyses were performed using Dr SPSS II for Windows (release 11.0.1J, IBM Japan, Inc., Tokyo, Japan) and R (The R Foundation for Statistical Computing), EZR on R commander (programmed by Y Kanda).

RESULTS

Incidence and onset of HLH

The cumulative incidence of HLH after SCT was 4.3% (24 out of 554). In 24 patients with HPS, 10 patients were male and 14 were female. The age of patients ranged from 4 to 64 years (median, 51 years). HPS occurred in seven patients with non-Hodgkin's lymphoma, four with AML, four with multiple myeloma, three with myelodysplastic syndrome, two with ALL, two with adult T-cell leukemia and one with CML and AA. The origin of stem cell was BMT in 10 patients, PBSC transplantation in 8 patients and CBT in 6 patients. A related donor was used for 3 patients, an unrelated donor was used for 15 patients and 6 patients underwent auto-transplantation. The median time from the date of transplant to onset of HLH was 21 days (range: 10–267 days). The onsets of many patients with HLH were before 30 days after SCT. Patients who had HLH after day +30 were 6 out of 24 patients. (day +36, +41, +49, +81, +175, +267). No patient was proved as evident viral infection.

Risk factors for HLH

Factors predicting HLH after transplantation were analyzed. Univariate analysis showed no difference in age at transplant, gender, original disease, stem cell source and donor between patients with HLH and no HLH (Table 1). In terms of the conditioning regimen, use of etoposide was the only factor that reduced HLH after SCT ($P = 0.027$). Other therapies, including BU, fludarabine, anti-thymocyte globulin and TBI, were not related to the occurrence of HLH. Moreover, reduced intensity conditioning or myeloablative condition was not associated with HLH. All three patients who developed complicated HLH despite the use of an etoposide-containing conditioning had undergone autologous transplantation and were alive at the time of analysis. Occurrence rate of HLH with or without etoposide is shown in Figure 1. In patients who had undergone SCT without etoposide-containing regimen, incidence of HLH was 6.26% at 1 year after SCT. On the other hand, incidence was 1.66% in those with etoposide-containing regimen, and they were statistically significant ($P = 0.0235$). Moreover, we performed Cox proportional hazards regression analysis treated death as a time-dependent covariate. This analysis revealed etoposide was statistically significant factor of HLH ($P = 0.03472$).

We also analyzed by onset day of HLH (early onset: under 30 days after SCT, late onset: over 30 days after SCT). In characteristics of patients, all six patients were female in the late onset group. However, other factors, age, original diseases, stem cell source, conditioning regimen and GVHD prophylaxis, were not different in the two groups. Survival rate of both groups was also not different.

Table 1. Comparison of patients with HLH and those without HLH

	HLH (n = *24*)	*no HLH* (n = *530*)	P-*value*
Age (median), years	51	48	0.785
Gender (M/F)	10/14	300/230	0.206
Disease			0.298
ALL	2	74	
AML	4	115	
NHL	7	160	
HL	0	13	
ATL	2	8	
MM	4	57	
AA	1	12	
Other	4	91	
Source			0.600
BM	10	209	
PB	8	224	
CB	6	97	
Donor			0.388
Autologous	6	182	
Allogeneic	18	348	
Related	3	123	0.130
Unrelated	15	225	
Conditioning			
BU	5	144	0.640
TBI	13	291	1.000
VP16	3	180	**0.027**
Flu	12	196	0.203
L-PAM	7	144	0.817
CY	11	277	0.677
ATG	1	9	0.360
CBDCA	3	40	0.287
MCNU	3	81	0.421
RIST	13	229	0.301
MAST	11	301	
GVHD prophylaxis in patients with allogeneic transplantation			
Cyclosporine	8	139	0.479
Tacrolimus	10	198	0.672
MTX	13	281	1.000
Methyl prednisolone	0	22	0.616
MMF	0	9	1.000
aGVHD (grade II–IV)	4	107	0.601

Abbreviations: AA = aplastic anemia; ATG = anti-thymocyte globulin; ATL = adult T-cell leukemia; CB = cord blood; CBDCA = carboplatin; F = female; Flu = fludarabine; HL = Hodgkin's lymphoma; HLH = hemophagocytic lymphohistiocytosis; L-PAM = melphalan; M = male; MCNU = methyl chloroethyl nitroso urea; MM = multiple myeloma; MMF = mycophenolate mofetil; MAST = myeloablative conditioning SCT; NHL = non-Hodgkin's lymphoma; PB = peripheral blood; RIST = reduced intensity conditioning SCT; VP16 = etoposide. Bold indicates $P < 0.05$.

Treatment and outcomes after HLH

Treatment analysis was performed using data from 23 patients with HLH (one patient was excluded because of insufficient data). Of these patients, 21 patients were treated with prednisolone or methylprednisolone. Four patients were treated with cyclosporine, two were treated with mycophenolate mofetil, one was treated with etoposide, and one was treated with foscarnet. Two patients underwent re-transplantation for the purpose of treating HLH. We compared factors at diagnosis of HLH between patients who were successfully treated and those who failed treated

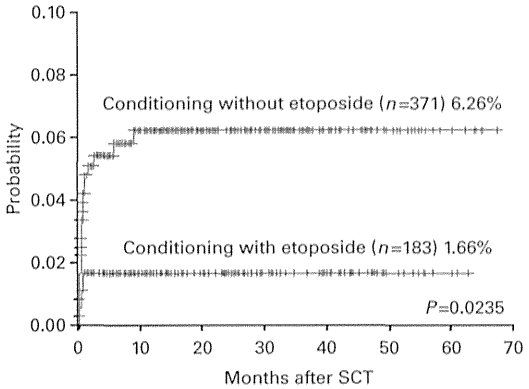

Figure 1. Occurrence rate of HLH with or without etoposide. In patients who had undergone SCT without etoposide-containing regimen, incidence of HLH was 6.26% at 1 year after SCT. On the other hand, incidence was 1.66% in those with etoposide-containing regimen and they were statistically significant ($P = 0.0235$).

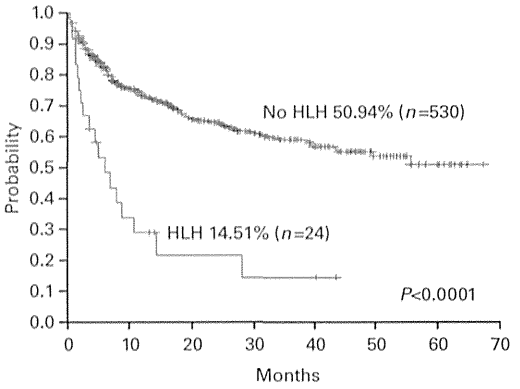

Figure 2. Overall survival curve for patients with and without HLH who received SCT. Patients without HLH had significantly higher overall survival (50.94% versus 14.91%) when compared with patients with HLH.

Table 2. Comparison of patients with HLH in which treatment was effective versus those in which treatment was not effective

	Effective (n = *14*)	*Non-effective* (n = *9*)	P-*value*
Age (median), years	53	45	0.231
Gender (M/F)	7/7	2/7	0.228
Donor (allo/auto)	8/6	9/0	**0.048**
Onset (days)	21	18	0.636
WBC ($\times 10^9$/L)	0.48	0.17	0.177
Plt ($\times 10^9$/L)	23	9	0.156
T.Bil (mg/dL)	0.65	2.0	**0.046**
AST (IU/L)	20	27	0.328
LDH (IU/L)	173.5	258.5	0.172
Ferritin (ng/mL)	2048	5340	0.165
PT (s)	11.4	13.2	**0.027**
Fib (mg/dL)	437	416	0.372
FDP (μg/mL)	5.05	16	**0.046**
Treatment			
Steroid hormone	13	8	1.000
Cyclosporine	0	4	**0.014**
MMF	0	2	0.142
Repeated SCT	1	1	1.000

Abbreviations: AST = aspartate aminotransferase; Fib = fibrinogen; FDP = fibrin/fibrinogen degradation products; LDH = lactate dehydrogenase; MMF = mycophenolate mofetil; PT = prothrombin time; T.Bil = total bilirubin; WBC = white blood cell. Bold indicates $P < 0.05$.

(Table 2). All patients who received autologous transplantation were successfully treated. Patients with liver dysfunction (for example, high total bilirubin level, prolonged prothrombin time and high level of fibrinogen degradation products) had a poor response to treatment for HLH. Cyclosporine was used in many patients with ineffective results. However, cyclosporine may be used because of resistance to steroid hormone. The survival rate was markedly lower in patients with HLH when compared with those who did not develop HLH (14.5% versus 50.9%, $P < 0.0001$, Figure 2). However, there was no difference between survival rate of successfully treated patients and that of failed patients (data not shown).

DISCUSSION

Various studies have described HLH occurring after autologous or allogeneic SCT.[6–24] The frequency of HLH after SCT was reported as 4.1% in patients who had undergone autologous and allogeneic SCT[6] and was reported as 16.8% in patients who underwent CBT.[7] In the present study, the cumulative incidence of HLH was 4.3% in all patients and was 5.8% in patients who underwent CBT.

Although some studies have described HLH occurring after SCT, there are few data regarding risk factors for HLH after SCT. Takagi *et al.*[7] reported that fewer infused CD34$^+$ cells are a significant risk factor for the development of HLH, and Aldelkefi *et al.*[6] reported that HLH after SCT was observed in many patients with allogeneic SCT and AA. Unfortunately, multicenter analysis did not include data on CD34+ cells in our study. Moreover, AA was not risk factor of HLH in our analysis. In our analysis, a conditioning regimen containing etoposide was the only factor associated with a reduction in HLH. Although HLH was observed in more patients who had undergone allogeneic SCT when compared with those who had undergone autologous SCT, this difference did not reach the level of statistical significance. Because etoposide is known as a therapeutic agent for HLH, we think it is very interesting that a conditioning regimen containing etoposide resulted in a reduction in the development of HLH after SCT.[8,9] The manuscript as risk factor including use of etoposide about HLH was never seen until now. The clear reason conditioning regimen containing etoposide controls development of HLH is not known. Most, but not all, studies suggest that the activated macrophage in patients with HLH is of donor origin.[9,10] Therefore, it is possible that an etoposide-containing condition regimen results in a decrease in macrophage activity, thereby reducing the risk of HLH. Previous studies of HLH after SCT have not included many patients who received an etoposide-containing condition regimen.[8,10–24] Indeed, none of the patients in the report by Takagi *et al.* received an etoposide-containing condition regimen.[6] These facts may also serve to support our speculation. Moreover, all three patients who developed complicated HLH despite the use an etoposide-containing condition regimen were alive at the time of analysis. Although all of these patients underwent autologous transplantation, use of etoposide as conditioning agent may improve the effectiveness of treatment for HLH after SCT.

In our analysis, many patients were treated with steroids. Etoposide, which is an effective therapeutic agent for HLH, was used in only one patient. However, treatment was more effective in patients who underwent autologous transplantation and in those with normal hepatic function. In patients who underwent autologous transplantation, HLH were known as good prognosis compared with patients who underwent allogeneic transplantation. However, the grade of liver dysfunction was not known as a prognostic factor in the treatment of HLH. In patients with HLH,

liver dysfunction is likely to progress. Therefore, early diagnosis and treatment is very important in patients with HLH.

Although about half of patients had a favorable response to steroids, the overall survival rate in patients with HLH was statistically lower than that for patients without HLH. It is possible that administration of steroids depresses GVHD and thereby increases the risk of relapse.

Some viruses (for example, CMV, Epstein–Barr virus) may be associated with HLH after SCT. Although, none of the patients developed viral infections in the present study, we cannot exclude the possibility that certain viruses are related to the risk of HLH. Regardless, etoposide-containing conditioning regimens will be effective in preventing HLH after SCT.

In conclusion, we find that etoposide-containing conditioning regimens reduce the occurrence of HLH after SCT. Other risk factors for HLH after SCT were not identified. Physicians should be cautious of HLH, while not using etoposide for conditioning regimen.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Pretransplant administration of imatinib for allo-HSCT in patients with *BCR-ABL*–positive acute lymphoblastic leukemia

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Key Points

- Pretransplant imatinib improved both relapse and nonrelapse mortality in patients with *BCR-ABL*–positive acute lymphoblastic leukemia.

We aimed to evaluate the impact of pretransplant imatinib administration on the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in adults with Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL). We retrospectively analyzed 738 patients with Ph^+ ALL that underwent allo-HSCT between 1990 and 2010 using data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation. We compared the allo-HSCT outcomes between 542 patients who received imatinib before allo-HSCT during the initial complete remission period (imatinib cohort) and 196 patients who did not receive imatinib (non-imatinib cohort). The 5-year overall survival after allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (59% vs 38%; 95% confidence interval [CI], 31-45%; $P < .001$). Multivariate analysis indicated that pretransplant imatinib administration had beneficial effects on overall survival (hazard ratio [HR], 0.57; 95% CI, 0.42-0.77; $P < .001$), relapse (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$), and nonrelapse mortality (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). In conclusion, our study showed that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of allo-HSCT in patients with Ph^+ ALL. (*Blood.* 2014;123(15):2325-2332)

Introduction

The treatment of Philadelphia chromosome–positive (Ph^+) acute lymphoblastic leukemia (ALL) has changed dramatically since the introduction of imatinib. Most imatinib-treated patients achieve complete remission (CR), and hematopoietic stem cell transplantation (HSCT) can be performed in a substantial proportion of patients who have achieved major or complete molecular remission.[1-4] Several studies have shown improvements in overall survival (OS) since the incorporation of imatinib-based therapy.[5-9] However, the possible benefits of imatinib administration before HSCT have not been extensively examined. In Japan, imatinib was initially used to treat Ph^+ ALL in the Japan Adult Leukemia Study Group (JALSG) ALL202 study, which began in February 2002, and has been widely used since 2005.[4] A comparison of the clinical outcomes of the 60 patients enrolled in the JALSG ALL202 study with those of patients from the pre-imatinib era strongly suggested that Ph^+ ALL patients who received imatinib before allogeneic HSCT (allo-HSCT) during the initial CR period had significantly improved OS compared with those who did not receive imatinib.[10] In the present study, we used data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation (JSHCT) to perform a large retrospective analysis of the clinical impact of imatinib administration before allo-HSCT.[11,12]

Methods

Data source and patient selection criteria

For this retrospective observational study, patient data were provided by the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network.[11] In the Transplant Registry Unified Management Program, patient survival, disease status, and long-term complications, including chronic

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Table 1. Characteristic of 738 patients with Ph$^+$ ALL who received allo-SCT

Characteristic	Non-imatinib cohort (n = 196)	Imatinib cohort (n = 542)	*P*
Age at SCT, years (%)			
≤29	48 (24)	99 (18)	
30-54	140 (71)	365 (67)	
≥55	8 (4)	78 (14)	
Median	39	42	<.001
Gender (male/female)	109/87	293/249	.708
Donor status (%)			
Related	121 (62)	178 (33)	
Unrelated	75 (38)	364 (67)	<.001
HLA disparity (%)			
Matched	139 (71)	330 (61)	
Mismatched	56 (29)	211 (39)	
Unknown	1 (0)	1 (0)	.010
Stem cell source (%)			
Bone marrow	151 (77)	345 (64)	
Peripheral blood	36 (18)	86 (16)	
Cord blood	9 (5)	111 (20)	<.001
PS at SCT (%)			
0	70 (36)	311 (57)	
1-4	45 (23)	218 (40)	
Unknown	81 (41)	13 (2)	.632
Days from diagnosis to SCT (%)			
<180	94 (48)	286 (53)	
≤180	98 (50)	255 (47)	
Unknown	4 (2)	1 (0)	.352
BCR-ABL subtype (%)			
Major	24 (12)	70 (13)	
Minor	69 (35)	352 (65)	
Major and minor	1 (0)	18 (3)	
Unknown	102 (52)	102 (19)	.039
Donor recipient gender match (%)			
Male-male	43 (22)	180 (33)	
Male-female	38 (19)	129 (24)	
Female-male	35 (18)	97 (18)	
Female-female	33 (17)	111 (20)	
Unknown	47 (24)	25 (5)	.463
Conditioning regimen (%)			
Reduced intensity	1 (1)	44 (8)	
Myeloabrative	121 (62)	479 (88)	
Unknown	74 (38)	19 (4)	<.001
WBC at diagnosis (%)			
<30 000/μL	109 (56)	288 (53)	
≤30 000/μL	74 (38)	247 (46)	
Unknown	13 (7)	7 (1)	.178
GVHD prophylaxis (%)			
CyA/methotrexate	133 (68)	228 (42)	
Tacrorimus/methotrexate	46 (23)	266 (49)	
Other/unknown	17 (9)	48 (9)	<.001
Cytogenetics (%)			
t(9;22) only	180 (92)	461 (85)	
Other abnormality	16 (8)	81 (15)	.016
ABO blood type disparity (%)			
Match	65 (33)	266 (49)	
Minor	24 (12)	119 (22)	
Major	37 (19)	152 (28)	
Unknown	70 (36)	5 (1)	.747
Transplant year (%)			
1990-2005	183 (93)	139 (26)	
2006-2010	13 (7)	403 (74)	<.001
MRD status at SCT			
Positive	44 (22)	144 (27)	
Negative	23 (12)	256 (47)	<.001

CyA, cyclospoline.

graft-versus-host disease (GVHD) and secondary malignancies, are reviewed annually using follow-up forms.[12] Ph$^+$ ALL was diagnosed by the presence of the *Ph* chromosome using cytogenetics and/or fluorescence in situ hybridization analysis and the determination of *BCR-ABL* fusion transcript positivity via real-time quantitative polymerase chain reaction (PCR) analysis. Grafts from unrelated donors were exclusively bone marrow derived because peripheral blood stem cell donation from unrelated donors was not approved in Japan during the study period. The timing and procedure of allo-HSCT, including the conditioning regimens, GVHD prophylaxis, and *BCR-ABL* transcript level assessments, were determined at each institution. *BCR-ABL* transcript levels were not compensated with a correction factor. In most laboratories, *BCR-ABL* mRNA copy numbers were normalized relative to glyceraldehyde-3-phosphate dehydrogenase mRNA copy numbers and expressed as copies per microgram of RNA. The quantification threshold was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}; nondetection of *BCR-ABL* or samples below this threshold was designated as "not detected" or "<50 copies/μg" (presented herein as PCR negative). Minimum residual disease (MRD) was evaluated using real-time quantitative PCR within a 30-day period before transplantation. Therapeutic decisions regarding tyrosine kinase inhibitor (TKI) administration after allo-HSCT were made at each institution. This study was approved by the data management committees of the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network and by the Institutional Review Board of the Fujita Health University. This study was conducted in accordance with the Declaration of Helsinki.

Patient selection

To attain an adequate level of comparability in terms of the allo-HSCT regimens, the following inclusion criteria were used: (1) presence of de novo Ph$^+$ ALL; (2) age of 16 to 59 years; (3) allo-HSCT during the first CR; and (4) initial HSCT between 1990 and 2010. Additional data on pretransplant imatinib administration and MRD at the time of allo-HSCT were also collected for this study. Of the 865 patients who fulfilled these criteria, information on pretransplant imatinib administration was available for 739 patients. One patient was excluded because of missing information on the date of relapse. Finally, 738 patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR were analyzed.

Statistical considerations

The primary end point of our study was OS after allo-HSCT. Secondary end points included the incidence of nonrelapse mortality (NRM) and relapse. The observation periods for OS were calculated from the date of transplantation until the date of the event or the last known date of follow-up. The OS probabilities were estimated according to the Kaplan-Meier product limit method. The cumulative relapse and NRM incidences were estimated while considering the competing risk, as described elsewhere.[13] For each estimate of the cumulative event incidence, death without an event was defined as a competing risk. Risk factors were evaluated using a combination of univariate and multivariate analyses. The following variables were evaluated: imatinib use before HSCT (yes vs no), age group in years (40-54 and 55-59 vs <40), donor and stem cell source (bone marrow from unrelated donor, peripheral blood from related donor or cord blood vs bone marrow from related donor), human leukocyte antigen (HLA) disparity (matched [HLA identical siblings or 6/6 allele-matched unrelated] vs mismatched), performance status (PS) at allo-HSCT (0 vs 1-4), time from diagnosis to allo-HSCT (<180 vs ≥180 days), BCR-ABL subtype (major vs minor vs major and minor), donor-recipient gender match (male-male vs male-female vs female-male vs female-female), conditioning regimen (decreased intensity vs myeloablative), white blood cell (WBC) count at diagnosis (<30 000/μL vs ≥30 000/μL), GVHD prophylaxis (CyA/methotrexate vs tacrolimus/methotrexate), cytogenetics [t(9;22) only vs more/other abnormalities], and ABO blood type compatibility (match, minor mismatch, or major mismatch). Continuous CR was defined as the absence of any hematological recurrence. We defined the following dosages as decreased-intensity regimens: busulfan, <9 mg/kg; melphalan, ≤140 mg/m^2; and total body irradiation, <500 cGy (single or fractionated) or 500 to 800 cGy (fractionated).[14] Donor and recipient pairs were considered matched when the

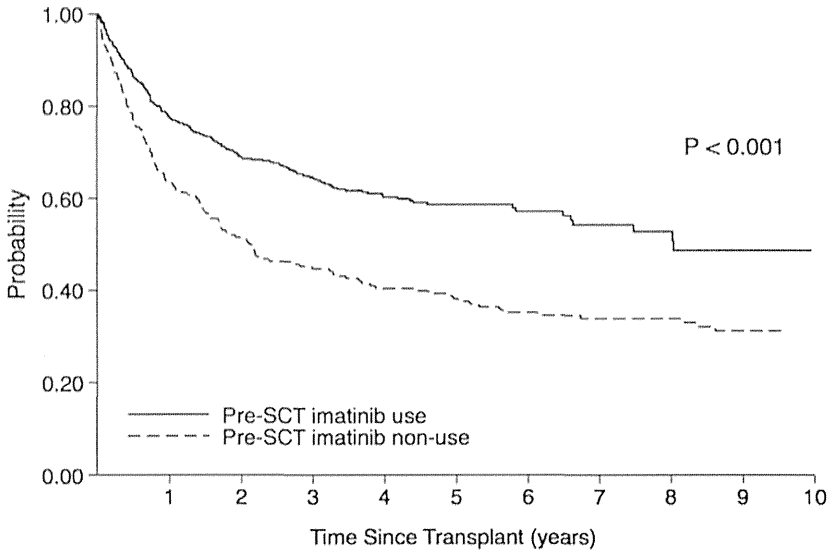

Figure 1. Effects of imatinib administration before stem cell transplantation on the overall survival of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period.

HLAs were matched at the A, B, and DRB1 loci, as determined by low-resolution HLA typing in allo-SCT from a related donor or cord blood. For unrelated allo-HSCT, matching at the HLA-A, B, Cw, and DRB1 loci in HLA high-resolution molecular typing was considered matched cases. Mismatches were defined by the presence of ≥1 disparity among these loci. Univariate analysis was performed using Cox regression models or a log-rank analysis. Multivariate analysis was performed using the Cox proportional hazards regression model or the competing risk regression model,[15] as appropriate. Demographic differences among groups were evaluated using the χ^2 or Wilcoxon rank-sum tests as appropriate. All statistical analyses were performed with STATA 11 software (STATA Corp., College Station, TX).

Results

Patient characteristics

The 738 study patients included 402 men and 336 women with a median age of 41 years (range, 16-59 years). HLA matching information was not available for 2 patients. The donor sources included HLA-identical sibling donors (n = 280), unrelated donors (n = 439), and other related donors (n = 19). There were no significant differences between the imatinib and non-imatinib cohorts with respect to gender, PS at allo-HSCT, interval between diagnosis and allo-HSCT, donor-recipient gender match, WBC count at diagnosis, or donor-recipient ABO compatibility, whereas significant differences were observed with respect to the age distribution at allo-HSCT, donor status, HLA disparity, stem cell source, BCR-ABL subtype, conditioning regimen, GVHD prophylaxis, and cytogenetics (Table 1). Of the 196 patients in the non-imatinib cohort, 183 (93%) underwent allo-HSCT between 1990 and 2005. In contrast, 403 of the 542 (74%) patients in the imatinib cohort underwent allo-HSCT between 2006 and 2010.

Outcomes

Overall survival. The median follow-up duration of the allo-HSCT survivors was 1551 days (range, 66-6648 days), and the 3- and 5-year OS rates for all patients were 59% (95% confidence interval [CI], 55-63%) and 53% (95% CI, 49-56%), respectively. The 5-year OS in the imatinib cohort was 59% (95% CI, 54-63%), which was significantly higher than that in the non-imatinib cohort (38%; 95% CI, 31-45%; $P < .001$; Figure 1). Table 2 shows the OS risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on OS, as revealed by univariate analysis (hazard ratio [HR], 0.56; 95% CI, 0.45-0.70; $P < .001$] and confirmed by multivariate analysis (HR, 0.57; 95% CI, 0.42-0.77; $P < .001$). In addition, age, interval between diagnosis and HSCT, and WBC count at diagnosis were significant prognostic factors for OS in the multivariate analysis.

Relapse. Relapse after allo-HSCT occurred in 116 (21%) and 66 (34%) patients in the imatinib and non-imatinib cohorts, respectively, after median periods of 232 (range, 19-2560 days) and 258 days (range, 42-2350 days), respectively. In the imatinib cohort, the estimated 3-year cumulative incidence of relapse was 23% (95% CI, 20-27%), which was significantly lower than that in the non-imatinib cohort (39%; 95% CI, 31-47%; $P < .001$; Figure 2). Table 3 shows the relapse risk factor analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on relapse, as determined by univariate analysis (HR, 0.52; 95% CI, 0.39-0.71; $P < .001$) and confirmed by multivariate analysis (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$). In addition, the following were significant prognostic factors for relapse: age, 30 to 54 years; HLA disparity; and female-male donor-recipient matching.

NRM. Overall, 207 (38%) patients in the imatinib cohort and 131 (67%) in the non-imatinib cohort died after allo-HSCT within median periods of 178 (range, 8-2935 days) and 177 days (range, 5-4549 days), respectively. Of these, 124 (23%) and 71 (36%) deaths in the former and latter cohorts, respectively, were not related to relapse after allo-HSCT. The major causes of all deaths and their respective frequencies in the imatinib and non-imatinib cohorts were as follows: relapse (40% vs 47%), infection (18% vs 9%), organ failure (12% vs 9%), interstitial pneumonia (6% vs 4%), GVHD (5% vs 9%), transplantation-associated thrombotic microangiopathy (2% vs 2%), bleeding (2% vs 5%), sinusoidal obstruction syndrome (1% vs 5%), and others (13% vs 11%). The estimated cumulative incidence of NRM at 3 years was significantly lower in the imatinib cohort (22%; 95% CI, 18-26%) than in the non-imatinib cohort (30%; 95% CI, 24-37%; $P = .002$; Figure 2). Table 4 shows the NRM risk factor

Table 2. Results of univariate and multivariate analysis of overall survival among 738 patients with Ph^+ ALL

	Univariate analysis		Multivariate analysis	
Variable	RR (95% CI)	P	RR (95% CI)	P
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.56 (0.45-0.70)	<.001	0.57 (0.42-0.77)	<.001
Age at SCT (regression)	1.02 (1.01 −1.03)	.002	1.02 (1.01-1.03)	<.001
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.89 (0.71-1.11)	.30	0.89 (0.66-1.20)	.430
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	0.81 (0.62-1.10)	.13	0.92 (0.63-1.33)	.640
Related peripheral blood	1.08 (0.79-1.48)	.64	1.27 (0.90-1.78)	.180
Cord blood	0.86 (0.61-1.22)	.39	1.25 (0.78-2.0)	.360
PS at SCT				
0	1 (Reference)	.24	1 (Reference)	
1-4	1.15 (0.91-1.47)		1.05 (0.82-1.36)	.690
Duration from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.26 (1.02-1.57)	.03	1.31 (1.03-1.67)	.030
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.77 (0.36-1.66)	.51	NA	
Major and minor	0.90 (0.44-1.83)	.78		
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.61-1.11)	.21	0.78 (0.57-1.06)	.110
Female-male	0.78 (0.56-1.08)	.13	0.77 (0.55-1.07)	.120
Female-female	0.71 (0.51-0.98)	.03	0.70 (0.50-0.98)	.040
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.96 (0.60-1.53)	.87	1.04 (0.64-1.70)	.150
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.29 (1.04-1.61)	.02	1.07 (0.99-1.14)	.053
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.78 (0.62-0.98)	.03	0.98 (0.73-1.31)	.899
Cytogenetics				
t(9;22)only	1 (Reference)		1 (Reference)	
Other abnormality	0.97 (0.71-1.34)	.87	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.12 (0.83-1.51)	.48	NA	
Major	1.21 (0.93-1.59)	.16	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

analysis. Imatinib administration before allo-HSCT had a significantly favorable effect on NRM, as determined by univariate (HR, 0.65; 95% CI, 0.49-0.88; $P < .001$) and multivariate analyses (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). Age was also found to be a significant prognostic factor in multivariate analysis.

MRD. Data regarding MRD status before allo-HSCT were available for 67 (34%) patients in the non-imatinib cohort and 400 (74%) patients in the imatinib cohort (Table 1). Among the 467 patients, the MRD negativity rate before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (64%; 95% CI, 59-69% vs 34%; 23-47%; $P < .001$). The estimated cumulative incidence of relapse at 3 years was significantly lower in the MRD-negative patients than in the MRD-positive patients (20%; 95% CI, 15-25% vs 32%; 95% CI, 25-40%, respectively; $P = .0017$), and this tendency was significant in the imatinib cohort (19%; 95% CI, 15-25% vs 34%; 95% CI, 25-42%, respectively; $P = .0016$), but not in the non-imatinib cohort (27%; 95% CI, 10-48% vs 28%; 95% CI, 15-43%, respectively; $P = .566$). There was no significant difference in NRM between the MRD-negative and MRD-positive patients (19%; 95% CI, 14-24% vs 22%; 95% CI, 16-28% at 3 years, respectively; $P = .0642$).

Discussion

Although many studies have confirmed the beneficial effects of imatinib on the clinical outcomes of patients with Ph^+ ALL,[1-6] the potential benefits of pretransplant imatinib administration has not been investigated in a sufficient number of patients. In our study, which is the largest of its type to date, we analyzed the records of 738 patients during a long-term follow-up period to analyze the benefits of pretransplant imatinib administration in patients with Ph^+ ALL. We observed significant improvements in the relapse rate and NRM in patients who received imatinib before allo-HSCT compared with those who did not receive imatinib (23% vs 39%; $P < .001$ and 22% vs 30%; $P = .002$, respectively). In the MVA, pretransplant imatinib administration was shown to have a significant favorable effect on both relapse and NRM after allo-HSCT.

Some investigators have reported that MRD before HSCT can serve as a powerful predictor of a lower relapse rate. In an analysis of the outcomes of 95 patients with Ph^+ ALL who received pretransplant imatinib-based therapy, Lee et al showed that the strongest predictor of relapse was the patient's MRD status at the end of 2 courses of pretransplant imatinib-based chemotherapy.[16] In the present study, patients who were MRD negative before HSCT had a significantly lower relapse rate after HSCT compared with those who were initially MRD positive (20.0% vs 32%, $P = .0017$), and this tendency was remarkable in the imatinib cohort (19% vs 34%, $P = .0016$). Moreover, the MRD negativity rate for *BCR-ABL* patients before allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (62% vs 37%, $P < .001$). These data suggest that in the imatinib cohort, the powerful antileukemia activity associated with pretransplant imatinib administration extensively decreased the MRD before allo-HSCT and prevented subsequent relapse after allo-HSCT.

The Ph chromosome is an adverse prognostic factor in patients with ALL, and only allo-HSCT offers a curative option for patients with Ph ALL. However, the probability of NRM in patients who undergo transplantation during the initial CR is relatively high; therefore, methods to decrease NRM were investigated. Recently, the UKALLXII/ECOG2993 study confirmed the superiority of allogeneic transplantation over chemotherapy on the basis of prospective outcome data from 267 unselected adult patients and reported that high NRM remained a significant problem in the pre-imatinib era.[17] Patient age, donor status, and HLA disparity are well-known prognostic factors for NRM after allo-HSCT.[1,3,17-19] In the present study, the risk of NRM was significantly lower in the imatinib cohort than in the non-imatinib cohort ($P = .002$), despite the former comprising significantly larger proportions of older recipients and unrelated and/or HLA-mismatched donors ($P < .001$, $P < .001$, and $P = .01$, respectively). Imatinib-based therapy has increased the proportion of patients who achieve sustained remission, thus providing additional

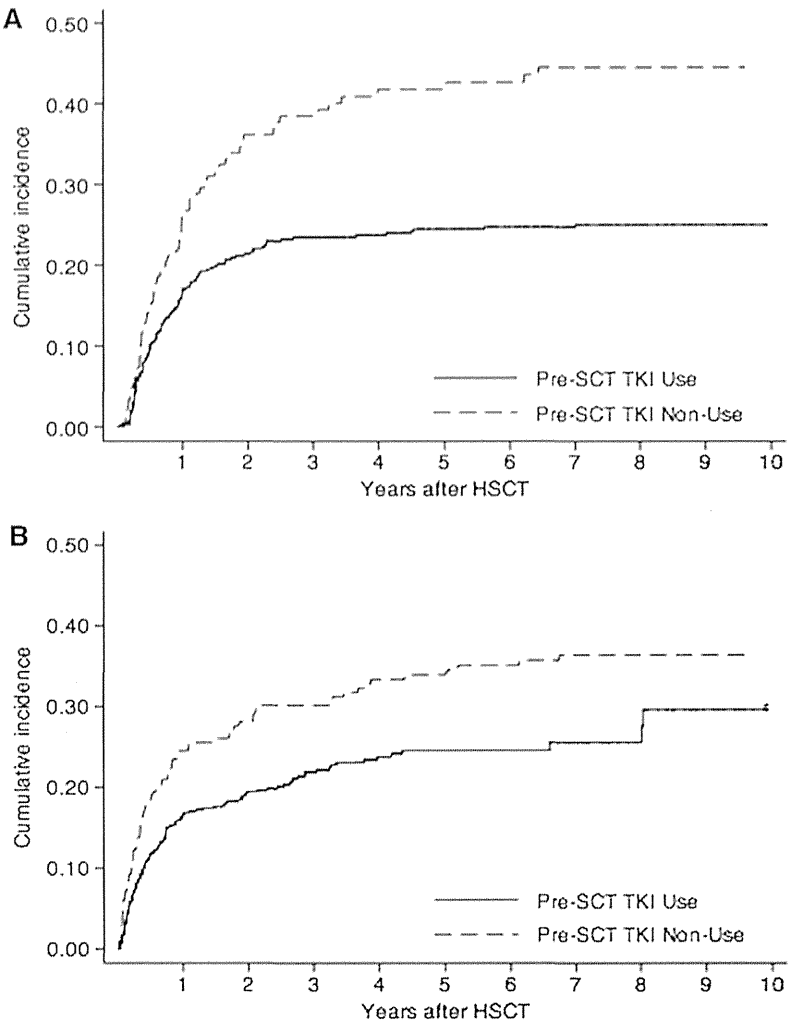

Figure 2. Cumulative incidence of relapse- or nonrelapse-related mortality of patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR period. (A) Relapse mortality. (B) NRM.

time for suitable donor selection and allo-HSCT and enabling individualized treatment approaches.[1-3] These secondary benefits may have contributed to the lower NRM in the imatinib cohort. Moreover, several recent studies have reported improved NRM following the incorporation or dose escalation of imatinib before allo-HSCT.[18-22] Given these findings, we believe that imatinib administration has allowed more patients with Ph$^+$ ALL to undergo allo-HSCT while in a better condition, resulting in the achievement of a lower NRM.

Over the last few decades, there have been many attempts to improve patient outcomes after allo-HSCT, including changes in the conditioning regimens and donor selection and the prophylaxis and treatment of organ complications, GVHD, and infectious diseases. In Japan, the period of 1990 to 2005 marked a pioneering era of cord blood transplantation, during which the relevance of cell doses and HLA matching had not yet been recognized. Laport et al reported their experiences with 79 patients with Ph$^+$ ALL who underwent allo-SCT with matched sibling donors; in these patients, the 5-year OS and NRM were examined according to the decade in which SCT was performed (1985-1995 vs 1996-2005), and no significant difference were observed between these 2 time periods.[23] In Japan, Kurosawa et al used a nationwide registry database of >6000 patients to retrospectively assess changes in the incidence and causes of NRM during 3 consecutive 4-year periods (1997-2000, 2001-2004, and 2005-2008).[24] The authors reported that the incidence of NRM after allo-HCT had significantly decreased during the entire 12-year period, which led to improvements in OS and decreases in NRM in subgroups comprising older patients (50-70 years of age) and/or those who received unrelated bone marrow transplants.[24] According to the present study, patients who underwent allo-HSCT with alternative donors and/or elderly patients would benefit from recent improvements in transplantation procedures, and this progress in transplantation may have partly contributed to the improved NRM in the imatinib cohort.

A strength of the present study was its large sample size; this permitted a more accurate estimation of the end points and added statistical power to the analyses. However, because this was a retrospective multicenter study, our results may be susceptible to the disadvantages of any retrospective study, such as heterogeneity in the treatment strategies selected by the physicians. With regard to patient selection bias, changes in patient selection and transplantation procedures throughout the study period (1990-2010) should also be considered. In Japan, the widespread use of alternative donors after 2000 facilitated the extension of allo-HSCT eligibility. Furthermore, cord blood cells were more frequently used in the imatinib cohort (20%) than in the non-imatinib

Table 3. Results of univariate and multivariate analysis of relapse among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.52 (0.39-0.71)	<.001	0.66 (0.43-0.99)	.048
Age at SCT (years)				
≤29	1 (Reference)		1 (Reference)	
30-54	0.58 (0.42-0.81)	.001	0.63 (0.45-0.89)	.009
≥55	0.60 (0.36-1.03)	.062	0.71 (0.40-1.30)	.250
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	0.52 (0.3-0.74)	<.001	0.48 (0.29-0.80)	.005
Stem cell source				
Related bone marrow	1 (Reference)			
Unrelated bone marrow	0.50 (0.35-0.70)	<.001	0.76 (0.48-1.21)	.251
Related peripheral blood	0.76 (0.50-1.16)	.202	0.91 (0.58-1.42)	.670
Cord blood	0.51 (0.31-0.84)	.008	1.2 (0.58-2.5)	.618
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.038 (0.75-1.44)	.821	NA	
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	0.91 (.68-1.22)	.538	1.16 (0.84-1.61)	.366
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	0.61 (0.20-1.91)	.400	NA	
Major and minor	1.22 (0.44-3.39)	.703	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.95 (0.65-1.40)	.790	1.02 (0.69-1.52)	.908
Female-male	0.59 (0.37-0.93)	.024	0.51 (0.32-0.81)	.004
Female-female	0.49 (0.30-0.80)	.004	0.53 (0.33-0.87)	.013
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	1.15 (0.61-2.17)	.675	0.95 (0.51-1.79)	.864
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.39 (1.03-1.87)	.029	1.08 (1.00-1.16)	.057
GVHD prophylaxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	0.56 (0.40-0.77)	<.001	0.74 (0.50-1.09)	.135
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.01 (0.65-1.57)	.955	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	0.75 (0.49-1.16)	.199	NA	
Major	0.86 (0.59-1.24)	.413	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

cohort (5%). These discrepancies resulted in different donor status, HLA disparity, and stem cell source frequencies in the present study.

An important difference in the pretransplant chemotherapy regimens should also be noted. Although detailed information about pretransplant chemotherapy was not available, the majority of the non-imatinib cohort was likely treated according to the JALSG ALL93[25] or JALSG ALL97 protocols,[26] whereas most of the imatinib cohort was likely to be treated according to the JALSG ALL202 protocols,[4] in which the chemotherapeutic regimen was similar to that used in the earlier protocols, except for the use of imatinib, because these were widely used regimens in Japan during the study period. Therefore, the influence of pretransplant chemotherapy appears to be limited.

In conclusion, our study involving a large number of patients observed over a long-term follow-up period clearly demonstrates that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of patients with Ph$^+$ ALL. This finding encourages us to consider allo-HSCT for patients with Ph$^+$ ALL even during the imatinib era; however, we should continue to investigate

Table 4. Results of univariate and multivariate analysis of NRM among 738 patients with Ph$^+$ ALL

	Univariate analysis		Multivariate analysis	
Variable	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Imatinib use before SCT				
No	1 (Reference)		1 (Reference)	
Yes	0.65 (0.49-0.88)	<.001	0.55 (0.37-0.83)	.005
Age at SCT (years)				
≤29	1 (Reference)		1.03 (1.02-1.05)	<.001
30-54	1.77 (1.16-2.70)	.008	(Regression)	
≥55	2.54 (1.51-4.30)	<.001		
HLA disparity				
Matched	1 (Reference)		1 (Reference)	
Mismatched	1.15 (0.86-1.54)	.330	1.27 (0.87-1.87)	.219
Stem cell source				
Related bone marrow	1 (Reference)		1 (Reference)	
Unrelated bone marrow	1.47 (1.01-2.13)	.044	1.32 (0.76-2.32)	.327
Related peripheral blood	1.37 (0.87-2.14)	.174	1.55 (0.95-2.53)	.081
Cord blood	1.49 (0.93-2.38)	.097	1.59 (0.81-3.11)	.181
PS at SCT				
0	1 (Reference)		1 (Reference)	
1-4	1.04 (0.76-1.42)	.810	0.90 (0.64-1.26)	.542
Days from diagnosis to SCT				
>180 days	1 (Reference)		1 (Reference)	
≤180 days	1.60 (1.20-2.13)	.001	1.35 (0.97-1.88)	.075
BCR-ABL subtype				
Major	1 (Reference)		1 (Reference)	
Minor	1.20 (0.40-3.62)	.750	NA	
Major and minor	0.96 (0.33-2.76)	.940	NA	
Donor recipient gender match				
Male-male	1 (Reference)		1 (Reference)	
Male-female	0.83 (0.55-1.25)	.380	0.73 (0.48-1.12)	.150
Female-male	1.01 (0.67-1.51)	.970	1.07 (0.71-1.62)	.737
Female-female	0.95 (0.63-1.42)	.790	0.85 (0.55-1.30)	.446
Conditioning regimen				
Reduced intensity	1 (Reference)		1 (Reference)	
Myeloabrative	0.76 (0.44-1.31)	.328	0.93 (0.52-1.67)	.819
WBC at diagnosis				
<30 000/μL	1 (Reference)		1 (Reference)	
≤30 000/μL	1.04 (0.78-1.38)	.810	1.03 (0.94-1.14)	.468
GVHD profiraxis				
CyA/MTX	1 (Reference)		1 (Reference)	
Tacrorimus/MTX	1.19 (0.89-1.60)	.250	1.23 (0.84-1.81)	.287
Cytogenetics				
t(9;22) only	1 (Reference)		1 (Reference)	
Other abnormality	1.03 (0.68-1.54)	.900	NA	
ABO blood type disparity				
Match	1 (Reference)		1 (Reference)	
Minor	1.31 (0.89-1.92)	.170	NA	
Major	1.28 (0.91-1.82)	.160	NA	

CyA, cyclospoline; NA, not applicable; RR, relative risk.

alternative treatment options for patients who are not eligible for allo-HSCT because of older age and/or comorbidity. For example, in recent years, MRD monitoring has been increasingly used as an independent prognostic factor in response to a number of studies that have demonstrated its importance. Ravandi et al analyzed the clinical outcomes of patients with Ph^+ ALL treated with TKI combined chemotherapy without allogeneic SCT and demonstrated that the achievement of a major molecular response status at 3 months (and beyond) after treatment initiation was associated with a decreased likelihood of relapse and a longer OS.[27] Bachanova et al used data from the Center for International Bone Marrow Transplant Research to analyze 197 patients with Ph^+ ALL and reported that the achievement of a MRD-negative status may lead to a low relapse rate and prolonged survival in response to either myeloablative conditioning or decreased-intensity conditioning HSCT. They also reported that MRD status may be more helpful than a predefined age cutoff in guiding decisions regarding the conditioning intensity before allo-HSCT.[28] In the TKI era, the potential of MRD monitoring via PCR was demonstrated; this technique allows us to identify patients who would benefit from treatment intensification and to select continued therapy without transplantation in older patients with poorer conditions. In addition, recent studies have shown that imatinib therapy before autologous HSCT is also beneficial.[7,29] The clinical relevance of autologous HSCT in patients with Ph^+ ALL should also be investigated as an alternative stem cell source in the TKI era.

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Authorship

Contribution: S.M., S.N., K.I., and J.T. designed the study and wrote the manuscript; S.M., Y.A., and K. Matsuo performed the statistical analysis and interpreted the data; H.K., K.O., T.F., Y.O., K. Miyamura, S.T., and M.O. provided the patient data; and Y.A., R.S., Y.M., K.K., and H.S. collected the patient data.

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bjh research paper

Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan

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Summary

To evaluate haematopoietic stem cell transplantation (HSCT) in children and adolescents, we reviewed the records of 47 patients who were ≤18 years, had relapsed or refractory anaplastic large cell lymphoma, and received HSCT between 1990 and 2010. At HSCT, complete remission (CR) was less common in allogeneic HSCT recipients ($n = 24$) than in autologous HSCT recipients ($n = 23$) ($P = 0{\cdot}01$). The autologous and allogeneic HSCT groups differed in terms of 5-year event-free survival (EFS) (38% vs. 50%, $P = 0{\cdot}63$), cumulative incidence of progress or relapse (49% vs. 28%, $P = 0{\cdot}25$), and treatment-related mortality (12% vs. 25%, $P = 0{\cdot}40$). However, these differences were not significant. Patients with non-CR at autologous HSCT had a significantly lower EFS rate (14% vs. 48%, $P = 0{\cdot}03$). Conversely, although those with non-CR at allogeneic HSCT had a lower EFS rate, this was not significant (44% vs. 63%, $P = 0{\cdot}26$). Reduced-intensity conditioning regimens were used for three of the 16 allogeneic HSCTs received by patients with non-CR. These three patients achieved CR, surviving 32–65 months after HSCT. These results demonstrated that allogeneic HSCT might be a treatment option for patients who do not achieve CR through conventional chemotherapy.

Keywords: anaplastic large cell lymphoma, children, adolescents, haematopoietic stem cell transplantation, reduced-intensity conditioning.

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Anaplastic large cell lymphoma (ALCL) is rare in children, accounting for 10–15% of childhood non-Hodgkin lymphoma cases (Murphy, 1994). The event-free survival (EFS) rate is 65–75% in children and adolescents receiving a first-line strategy based on short-pulse chemotherapy over a period of 3–6 months (Brugières *et al*, 1998, 2009a; Seidemann *et al*, 2001; Le Deley *et al*, 2010). Accordingly, the relapse rate is approximately 30% in most study series. The treatment of relapsed and refractory ALCL remains a matter of debate. Patients with relapsed ALCL have a 30–60% chance of survival under current treatment strategies, which include high-dose chemotherapy with haematopoietic stem cell transplantation (HSCT) and long-term treatment with vinblastine (Brugières *et al*, 2000, 2009b; Williams *et al*, 2002; Mori *et al*, 2006; Woessmann *et al*, 2006; Stockklausner *et al*, 2008; Gross *et al*, 2010). In contrast, patients who experience ALCL progression during first-line chemotherapy have extremely poor outcomes (Woessmann *et al*, 2006) and autologous or allogeneic HSCT is required as the most appropriate therapy.

Some evidence is available regarding the roles of autologous and allogeneic HSCT in paediatric ALCL. However, data are limited to several HSCT case series and case reports. In particular, few reports have been published regarding allogeneic HSCT for paediatric ALCL. We previously reported a retrospective analysis of 26 paediatric patients with recurrent ALCL in Japan (Mori *et al*, 2006). In that study, only three of the eight patients who received autologous HSCT while in their second complete remission (CR) survived without further relapse. In contrast, all six patients who received allogeneic HSCT while in their second CR survived without further relapse. However, our previous study included too few patients for us to discuss the efficacy of HSCT for relapsed or refractory childhood ALCL.

In the present study, we sought to evaluate the efficacy of HSCT for relapsed or refractory ALCL in children and adolescents. We performed a further retrospective analysis of 47 patients who received autologous or allogeneic HSCT for relapsed or refractory ALCL between 1990 and 2010.

Patients and methods

Patients and transplantations

This study was approved by the institutional ethics committee of National Kyushu Cancer Centre. Data on patients who had undergone HSCT were collected from the registries belonging to the Transplant Registry Unified Management Program system of the Japan Society for Hematopoietic Cell Transplantation. The study included 47 patients who had a diagnosis of relapsed or refractory ALCL and received HSCT at age ≤18 years between March 1990 and September 2010. Twenty-three patients received autologous HSCT and 24 patients received allogeneic HSCT. Refractory disease was defined as progression during fist-line treatment. Reduced-intensity conditioning (RIC) regimens were defined as (a) total body irradiation of ≤500 cGy as a single fraction or ≤800 cGy if fractionated, (b) <9 mg/kg of busulfan, (c) ≤180 mg/m^2 of melphalan, (d) <10 mg/kg of thiotepa, or (e) the BEAM regimen (carmustine, etoposide, cytarabine and melphalan), according to previous reports (Yaniv & Stein, 2008; Giralt *et al*, 2009; Ohta *et al*, 2010; Luger *et al*, 2012). All other conditioning regimens were defined as myeloablative conditioning (MAC) regimens.

Statistical analysis

Overall survival (OS), EFS, cumulative incidences of relapse and treatment-related mortality (TRM) were estimated using the Kaplan–Meier method. The Mann–Whitney *U* test, χ^2-test, and Fisher's exact test were used to assess differences in patient characteristics. The level of statistical significance was set at $P < 0{\cdot}05$. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Autologous HSCT

The patients' characteristics are shown in Table I. Twenty-three patients received autologous HSCT for relapsed or refractory disease as their first transplantation. The median follow-up duration for survivors after autologous HSCT was 154 (range: 9–224) months. The median age at HSCT was 15 (range: 7–18) years. Sixteen patients had achieved CR at HSCT and seven patients had residual disease. Bone marrow and peripheral blood were the stem cell sources in three and 20 patients, respectively. Engraftment was observed in 23 (100%) cases, occurring at a median of 12 d. The 5-year cumulative incidence of relapse was 49% ± 11% (Fig 1A). Treatment-related death occurred in three of the patients who received autologous HSCT and the 5-year cumulative incidence of TRM was 12% ± 9% (Fig 1B). Two of the three patients died of infectious complications and one patient died of multiple organ failure. The 5-year OS and EFS rates were 51% ± 11% and 38% ± 10%, respectively (Fig 2A, B). We observed 5-year EFS rates of 48% ± 13% and 14% ± 13% for patients with CR and non-CR, respectively, at autologous HSCT (Fig 3A), which constituted a significant difference ($P = 0{\cdot}03$).

Allogeneic HSCT

Twenty-four patients received allogeneic HSCT for relapsed or refractory disease (Table I). The median follow-up duration for survivors after allogeneic HSCT was 68 (range: 32–212) months. The median age at HSCT was 13·5 (range: 3–18) years. Of the 24 patients, four had received previous autologous HSCT. Eight patients had achieved CR at HSCT and 16 patients had residual disease (Table I). The sources of stem cells were bone marrow in 13 patients, cord blood in