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ORIGINAL ARTICLE

Prognostic factors influencing clinical outcome of allogeneic hematopoietic stem cell transplantation following imatinib-based therapy in *BCR–ABL*-positive ALL

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We investigated prognostic factors for the clinical outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) following imatinib-based therapy. Among 100 adult patients who were prospectively enrolled in the JALSG Ph + ALL202 study, 97 patients obtained complete remission (CR) by imatinib-combined chemotherapy, among whom 60 underwent allo-HSCT in their first CR. The probabilities of overall survival (OS) and disease-free survival (DFS) at 3 years after HSCT were 64% (95% CI, 49–76) and 58% (95% CI, 43–70), respectively. Prognostic factor analysis revealed that the major *BCR–ABL* transcript was the only unfavorable predictor for OS and DFS after HSCT by both univariate (HR, 3.67 (95% CI 1.49–9.08); $P = 0.005$ and HR, 6.25 (95% CI, 1.88–20.8); $P = 0.003$, respectively) and multivariate analyses (HR, 3.20 (95% CI, 1.21–8.50); $P = 0.019$ and HR, 6.92 (95% CI, 2.09–22.9); $P = 0.002$, respectively). Minimal residual disease status at the time of HSCT had a significant influence on relapse rate ($P = 0.015$). Further study of the *BCR–ABL* subtype for the clinical impact on outcome of allo-HSCT in Ph + ALL is warranted.

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Keywords: philadelphia chromosome-positive acute lymphoblastic leukemia; imatinib; allogeneic hematopoietic stem cell transplantation; prognostic factor

INTRODUCTION

Approximately 20 to 25% of adult patients with acute lymphoblastic leukemia (ALL) harbor *BCR–ABL* fusion gene. The prognosis following conventional chemotherapy of these patients had been extremely poor.[1–3] Although the treatment of Philadelphia chromosome-positive ALL (Ph + ALL) has been changed dramatically since the introduction of imatinib,[4] allogeneic hematopoietic stem cell transplantation (allo-HSCT) still seems to have a central role as a curative option for patients with Ph + ALL in the imatinib era.[5–7] Previously we reported that the patients who had achieved complete remission (CR) by imatinib-based therapy, and subsequently received allo-HSCT in their first CR, showed significantly superior survival to those patients in the pre-imatinib era.[8] Imatinib-based therapy is a useful strategy, giving patients not only a better chance to receive allo-HSCT but also improvement of the outcome after allo-HSCT. However, the treatment success after allo-HSCT is impaired by the occurrence of post-transplant relapse and non-relapse mortality (NRM),[9–11] and therefore, identification of the risk factors causing relapse and NRM after allo-HSCT would be beneficial.

In the present study, we evaluated prognostic factors influencing overall survival (OS), disease-free survival (DFS), relapse and NRM after allo-HSCT among patients with Ph + ALL who underwent HSCT in the imatinib era, by using the prospectively conducted data of Japan Adult Leukemia Study Group (JALSG) Ph + ALL202 study.

PATIENTS AND METHODS

Patients

In the JALSG Ph + ALL202 study, 100 newly diagnosed patients with *BCR–ABL*-positive ALL were registered consecutively between September 2002 and May 2005. All patients were diagnosed as Ph + ALL by real-time quantitative PCR (RQ-PCR) analysis, and received the same imatinib-combined chemotherapy, as described previously.[12] Of 97 patients who achieved CR, 60 patients received allo-HSCT in their first CR. Table 1 shows the characteristics of these 60 patients analyzed in the present study.

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Table 1. Patient characteristics ($N = 60$)

Characteristics	*No. of patients*	*%*
Donor status		
Related	39	65
Unrelated	21	35
Age at HSCT (years)		
–39	33	55
40–	27	45
BCR–ABL isoform		
Minor	42	70
Major	18	30
Additional chromosome abnormality (4 subjects unknown)		
No	10	17
Yes	44	83
WBC at diagnosis ($\times 10^9$/l)		
<30	34	57
$\geqslant 30$	26	43
CD 20 positivity (9 subjects unknown)		
Negative	28	47
Positive	23	38
Stem-cell source		
Bone marrow	35	58
Peripheral blood	16	27
Cord blood	9	15
Conditioning regimen		
Myeloablative	54	90
CY + TBI	27	45
CY + CA + TBI	15	25
CY + VP + TBI	1	2
CY + TESPA + TBI	4	7
CY + BU	3	5
Others	4	7
Reduced intensity	6	10
Flu + BU	3	5
Flu + LPAM ± TBI	3	5
Performance status at HSCT		
0	39	65
1–2	21	35
MRD status at HSCT (3 subjects unknown)		
PCR negative	39	65
PCR positive	18	30
GVHD prophylaxis		
Cyclosporine + sMTX	31	52
Cyclosporine ± other	4	7
Tacrolimus + sMTX	23	38
Others	2	3

Abbreviations: BU, busulfan (oral); CA, cytarabine; CY, cyclophosphamide; Flu, fuludarabine; HSCT, hematopoietic stem-cell transplantation; LPAM, melphalan; MRD, minimal residual disease; sMTX, short-term methotrexate; TBI, total body irradiation; TESPA, tespamine; VP, etoposide; WBC, white blood corpuscles.

In the Ph + ALL202 study, allo-HSCT was recommended after achieving CR if a human leukocyte antigen (HLA)-identical donor was available. The stem-cell source for allo-HSCT was chosen in the following order: first, matched related donor; second, HLA-A, B and DRB1 allele matched (6/6) or DRB1 one-allele mismatched unrelated donor; and third, unrelated cord blood or HLA-mismatched related donor. Timing and procedure of HSCT, including conditioning regimen and graft-versus-host disease (GVHD) prophylaxis, were determined by each institution.

Among 60 patients, 32 were males and 28 females, with a median age of 37 years (range, 15–64 years), while 33 patients were less than 40. Regarding the *BCR–ABL* transcript types, two patients expressed both major and minor *BCR–ABL*s, and were categorized into the major *BCR–ABL* group in the subsequent analysis. Consequently, 42 patients were positive for minor *BCR–ABL* and 18 for major *BCR–ABL*. Pre-treatment cytogenetic results were not available for four patients because no analysis was performed ($n = 2$) nor successful ($n = 2$). Of the remaining 56 patients, 10 showed only t(9;22), 44 showed additional chromosome aberrations and 2 showed normal karyotype. Additional aberrations were comprised of + der (22) t(9;22) in 12 patients, del(9) in 3, monosomy 7 in 6 and trisomy 8 in 6. The study was approved by the institutional review board of each participating center and conducted in accordance with the Declaration of Helsinki.

Quantification of BCR–ABL Transcripts

The copy number of *BCR–ABL* transcripts in bone marrow was determined at the central laboratory using the RQ-PCR as described previously.[12] To minimize the variability owing to differences in the efficiency of cDNA synthesis and RNA integrity among patient samples, the copy numbers of *BCR–ABL* transcripts were converted to molecules per microgram RNA after being normalized by *GAPDH*. The normalized values of the *BCR–ABL* copies in each sample were reported as the *BCR–ABL* number of copies. At least 5.7×10^5 copies/μg RNA *GAPDH* levels were required in a sample to be defined as a negative PCR result; otherwise, the sample was not used for minimal residual disease (MRD) studies. The threshold for quantification was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}. The levels below this threshold were designated as 'not detected' or 'less than 50 copies/μg', and the former was categorized as PCR negativity. MRD at the time of HSCT was evaluated by the result of RQ-PCR within 30 days before respective transplantation.

Statistical Considerations

The aim of this study was to identify prognostic factors for clinical outcome after allo-HSCT in patients with Ph + ALL transplanted in their CR in the imatinib era. Primary endpoint was OS after allo-HSCT, and secondary endpoints were NRM, relapse and DFS. OS was calculated from the date of transplantation to the date of death by any cause, or the last known date of follow-up. DFS was computed from the date of transplantation to the date of relapse, or death by any cause, or the last known date of follow-up. The probabilities of OS and DFS were estimated by Kaplan–Meier product limit method. Cumulative incidence of NRM, relapse, acute GVHD (aGVHD) and chronic GVHD (cGVHD) were estimated by the method taking the competing risks into account, as described elsewhere.[13] In each estimation of cumulative incidence of events, death without an event was defined as a competing risk. Risk factors were evaluated by combination of uni- and multivariate analyses. We applied for univariate analysis Cox regression models or the log-rank test, and for multivariate analysis the Cox proportional hazards regression model or the competing risk regression model as appropriate.[14]

Covariates considered in uni- and multivariate analyses were: donor status, age at HSCT (<40, vs $\geqslant 40$), CD20 positivity (yes vs no), WBC counts at diagnosis ($>30 \times 10^9$/l vs $<30 \times 10^9$/l), additional chromosomal abnormality, stem-cell source (bone marrow, peripheral blood or cord blood), conditioning regimen (myeloablative vs reduced intensity), *BCR–ABL* subtype (major vs minor), performance status at HSCT (1–2 vs 0) and MRD at HSCT (PCR positive vs negative). Neutrophil recovery was defined by neutrophil counts of $\geqslant 0.5 \times 10^9$/l in three consecutive days. Graft failure was defined as no sign of neutrophil recovery. aGVHD and cGVHD were defined according to previously described standard criteria.[15]

RESULTS

Transplantation

Graft and conditioning regimen characteristics are summarized in Table 1. The median day from diagnosis to HSCT was 164 (range 67–512 days). One patient with no HLA-matched related donor received the scheduled therapy until a HLA-matched unrelated donor was available, and underwent HSCT at 512 days. The majority of donors were HLA-matched related ($n = 24$) and unrelated ($n = 21$), followed by mismatched unrelated cord blood ($n = 9$) and mismatched related donors ($n = 6$). Patients were

treated with various conditioning regimens according to the transplant centers. The majority of patients (70%) received fractionated total body irradiation followed by cyclophosphamide and/or cytarabine. Six patients, older than 55, were given a reduced-intensity regimen consisting of fludarabine and melphalan or busulfan. No patient received imatinib therapy after HSCT. All patients who showed hematological relapse after HSCT received salvage treatment comprising of imatinib and/or chemotherapy.

The median days to reach a neutrophil count $>0.5 \times 10^9$/l and platelet count $\geqslant 50 \times 10^9$/l were 15 (range: 5–41 days) and 27 (range: 11–504 days), respectively. Cumulative incidence of grade 2 to 4 of aGVHD and of cGVHD at 1 year after HSCT were 33.3% (95% CI, 12–33%) and 44% (95% CI, 29–58%), respectively.

OS and DFS

With a median follow-up of 31 months (range, 12 to 56) after HSCT, 41 patients were alive without relapse. The probability of OS and DFS at 3 years after HSCT were 64% (95% CI; 49–76%) and 58% (95% CI; 43–70%), respectively (Figure 1). By the uni- and multivariate analysis, the presence of major *BCR–ABL* transcript was only associated with unfavorable OS (HR = 3.67 (95% CI, 1.49–9.08); $P = 0.005$, and HR = 6.25 (95% CI, 1.88–20.8); $P = 0.003$, respectively) and DFS (HR = 2.60 (95% CI, 1.16–5.83); $P = 0.02$, and HR = 3.20 (95% CI, 1.21–8.50); $P = 0.019$, respectively) (Table 2). Figure 2 illustrates the 3-year OS and DFS in patients with major and minor *BCR–ABL* subtypes (37% vs 75%; $P = 0.003$ and 33% vs 68%; $P = 0.016$, respectively).

Relapse

Overall, 9 patients (15%) relapsed after HSCT, with a median day of 167 (range, 68–728 days). The estimated cumulative incidence of relapse at 3 years was 17% (95% CI, 8.3–28.0%). By the univariate analysis for relapse, PCR-negativity at HSCT (HR = 4.82 (95% CI, 1.20–19.4); $P = 0.027$) and peripheral blood as a stem-cell source (HR = 5.53 (95% CI, 1.06–29.0); $P = 0.043$) were associated with a lower relapse rate, but they did not reach statistical significance by the multivariate analysis (HR = 7.34 (95% CI, 0.54–99.4); $P = 0.134$ and HR = 4.92 (95% CI, 0.17–144.0); $P = 0.355$, respectively) (Table 3). The 3-year cumulative incidence of relapse rate was not different in patients with major and minor *BCR–ABL* subtypes (8% vs 20%; $P = 0.34$).

NRM

Nineteen patients died after HSCT: 6 from relapsed ALL and 13 from causes other than leukemia. The causes of NRM included graft failure in 5, infection in 3, bronchiolitis obliterans in 2, cGVHD in 2 and unknown in 1. Estimated cumulative incidences of NRM at 3 years were 26% (95% CI, 14.8–38.7). By both uni- and multivariate analyses, the presence of major *BCR–ABL* transcript was associated with a higher NRM rate (HR = 5.95 (95% CI, 2.06–17.2); $P = 0.001$, and 6.92 (95% CI, 2.09–22.9), vs 0.002, respectively) (Table 3).

Figure 2 illustrates the 3-year cumulative incidence of NRM in patients with major and minor *BCR–ABL* subtypes (57% vs 13%; $P = 0.0004$). Four patients (22%) with major *BCR–ABL* transcript, but only one (2%) with minor transcript, died from graft failure (Table 4).

DISCUSSION

In the present study, in patients with Ph + ALL who had achieved CR by imatinib-based therapy and subsequently received allo-HSCT in their first CR, the major *BCR–ABL* subtype revealed significantly unfavorable prognostic impact on NRM, and consequently on OS and DFS (Figure 2). During the pre-imatinib era, several groups reported the relationship between the clinical outcome and *BCR–ABL* subtypes in patients with Ph + ALL. German Multicenter Adult ALL Study Group reported a trend toward poor OS for patients with major *BCR–ABL* (19% OS for the minor and 3% for the major at 3 years, $P = 0.07$).[2] Gruppo Italiano Malattie Ematologiche dell' Adulto also reported that minor *BCR–ABL* was an independent prognostic factor favorably affecting the 5-year OS and DFS ($P = 0.008$ and $P = 0.02$, respectively), although response rates to the induction therapy were similar in both groups.[16] Of note in their study, none of 14 patients with major *BCR–ABL* transcript who underwent HSCT (8 allogeneic and 6 autologous) survived in CR, whereas, among 22 patients with minor *BCR–ABL*, 6 of 12 who received allo-HSCT and 2 of 10 who received autologous HSCT survived in CR.

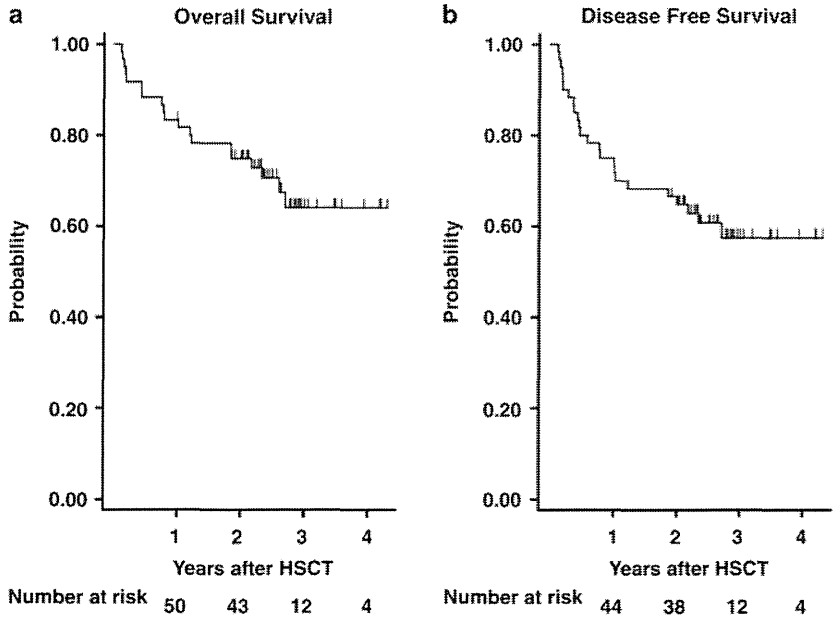

Figure 1. (**a**) OS and (**b**) DFS of 60 patients with Ph + ALL who underwent allo-HSCT in their first CR following imatinib-based therapy.

Table 2. Uni- and multivariate analyses for OS and DFS of 60 patients who received HSCT in their first CR following imatinib-based therapy

Characteristics	*OS*				*DFS*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Donor status								
Related	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Unrelated	1.43 (0.57–3.57)	0.443	1.27 (0.24–6.64)	0.779	0.93 (0.40–2.17)	0.865	0.72 (0.15–3.59)	0.692
Age at HSCT (years)								
–39	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
40–	1.55 (0.63–3.82)	0.339	3.04 (0.91–10.2)	0.072	1.09 (0.49–2.44)	0.833	1.22 (0.42–3.49)	0.715
Additional chromosome abnormality[a]								
No	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Yes	0.91 (0.26–3.20)	0.882	0.71 (0.17–3.02)	0.647	0.97 (0.33–2.89)	0.958	0.75 (0.21–2.72)	0.666
Stem-cell source								
Bone marrow	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Peripheral blood	0.73 (0.24–2.28)	0.592	1.19 (0.23–6.20)	0.840	1.58 (0.64–3.87)	0.318	1.99 (0.48–8.15)	0.340
Cord blood	1.01 (0.28–3.57)	0.994	2.61 (0.37–18.4)	0.335	1.52 (0.49–4.72)	0.468	1.94 (0.32–11.8)	0.473
Conditioning regimen								
Myeloablative	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Reduced intensity	NE		NE		NE		NE	
BCR–ABL subtype								
Minor	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Major	3.67 (1.49–9.08)	0.005	6.25 (1.88–20.8)	0.003	2.60 (1.16–5.83)	0.020	3.20 (1.21–8.50)	0.019
Performance status at HSCT								
0	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
1–2	1.90 (0.77–4.68)	0.165	0.91 (0.26–3.12)	0.879	1.81 (0.81–4.04)	0.148	1.55 (0.53–4.53)	0.423
MRD status at HSCT[a]								
PCR negative	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
PCR positive	1.32 (0.52–3.35)	0.562	1.12 (0.33–3.83)	0.860	1.47 (0.64–3.36)	0.361	1.27 (0.46–3.48)	0.642
WBC at diagnosis ($\times 10^9$/l)								
<30	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
$\geqslant 30$	1.50 (0.61–3.70)	0.376	1.44 (0.38–5.37)	0.590	1.71 (0.77–3.82)	0.191	1.67 (0.56–5.04)	0.360
CD 20 positivity								
Negative	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Positive	0.56 (0.20–1.54)	0.260	0.30 (0.08–1.21)	0.091	0.74 (0.30–1.84)	0.519	0.68 (0.20–2.36)	0.548

Abbreviations: CI, confidence of interval; DFS, disease-free survival; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; NE, not estimated; OS, overall survival; WBC, white blood corpuscles. [a]Subjects with unknown status were included in the analyses as dummy variable.

In this imatinib era study, patients with major *BCR–ABL* transcript showed significantly unfavorable OS rates, compared with those with minor *BCR–ABL* transcript. Among 100 patients registered into the JALSG Ph + ALL 202 study, three patients died from chemotherapy-related toxicity during induction therapy and all of them expressed minor *BCR–ABL* transcript. Additionally, among 40 patients who did not receive HSCT in their first CR, OS of 7 patients with major *BCR–ABL* transcript was not inferior to that of 33 patients with minor *BCR–ABL* ($P = 0.254$) (Supplemental figure S1). Therefore, the unfavorable clinical impact of major *BCR–ABL* transcript might be specific in the setting of allo-HSCT. Then the question arises: How the *BCR–ABL* subtype influenced the prognosis after allo-HSCT?

As shown in Table 4, MRD status and the period from diagnosis to HSCT were not significantly different among patients with major or minor *BCR–ABL* transcript. As the cause of NRM after allo-HSCT, high incidence of graft failure (22%) was observed in patients with major *BCR–ABL* (Table 4), and to predict NRM, transplantation-specific comorbidity index (HCT-CI) is reportedly useful.[17] In the present study, 54 of 60 patients could be evaluable for this scoring system, but we found no difference in HCT-CI scores between major and minor *BCR–ABL* subtypes ($P = 0.40$).

Biological heterogeneities between major and minor *BCR–ABL* transcripts may have influenced NRM of HSCT. Juric *et al.*[18] performed a comprehensive analysis of the gene expression profiles in 37 *BCR–ABL*-positive adult ALL. They identified the genes overexpressed (PILRB, STS-1, SPRY1) or underexpressed (TSPAN16, ADAMTSL4) in ALL with minor *BCR–ABL* transcript, relative to ALL with major *BCR–ABL*, and constructed a gene expression- and interaction-based outcome predictor, consisting of 27 genes, which correlated with OS, independent of age and WBC count at presentation. Zheng *et al.*[19] spotlighted the role of the reciprocal *ABL–BCR* fusion proteins, derivative chromosome 9 (der 9) -associated $p96^{ABL-BCR}$ and $p40^{ABL-BCR}$ fusion proteins. They indicated that $p96^{ABL-BCR}$ and $p40^{ABL-BCR}$ fusion proteins regulated the different expression of genes involved in the maintenance of stem-cell capacity. However, even if the biological heterogeneity would affect the clinical outcome of patients,

Table 3. Uni- and multivariate competing risk regression analyses for relapse and NRM of 60 patients who received HSCT in their first CR following imatinib-based therapy

Characteristics	*Relapse*				*NRM*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*	
	RR (95% CI)	P	*RR (95% CI)*	P	*RR (95% CI)*	P	*RR (95% CI)*	P
Donor status								
Related	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Unrelated	0.24 (0.03–1.92)	0.179	0.15 (0.01–2.51)	0.186	2.05 (0.74–5.69)	0.169	0.94 (0.24-3.63)	0.929
Age at HSCT (years)								
–39	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
40–	0.68 (0.16–2.84)	0.600	0.07 (0.04–1.28)	0.073	1.29 (0.42–3.95)	0.634	2.47 (0.56-10.8)	0.229
Additional chromosome abnormality[a]								
No	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Yes	1.21 (0.15–9.59)	0.858	4.53 (0.28–73.4)	0.288	0.75 (0.22–2.61)	0.655	0.68 (0.12-3.90)	0.666
Stem-cell source								
Bone marrow	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Peripheral blood	5.53 (1.06–29.0)	0.043	4.92 (0.17–144.0)	0.355	0.41 (0.10–1.73)	0.223	0.77 (0.11-5.23)	0.788
Cord blood	4.44 (0.68–29.2)	0.121	0.34 (0.01–10.1)	0.537	0.81 (0.16–4.09)	0.795	1.01 (0.10-9.89)	0.996
Conditioning regimen								
Myeloablative	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Reduced intensity	NE		NE		NE		NE	
BCR–ABL subtype								
Minor	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
Major	0.37 (0.05–2.90)	0.345	0.23 (0.05–1.15)	0.074	5.95 (2.06–17.2)	0.001	6.92 (2.09-22.9)	0.002
Performance status at HSCT								
0	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
1–2	1.67 (0.45–6.20)	0.442	6.88 (0.96–49.1)	0.054	1.47 (0.52–4.14)	0.470	1.03 (0.24-4.46)	0.972
MRD status at HSCT[a]								
PCR negative	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
PCR positive	4.82 (1.20–19.4)	0.027	7.34 (0.54–99.4)	0.134	0.57 (0.15–2.13)	0.402	0.75 (0.15-3.84)	0.732

Abbreviations: CI, confidence interval; CR, complete remission; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; NE, not estimated; NRM, non-relapse mortality. [a]Subjects with unknown status were included in the analyses as dummy variable.

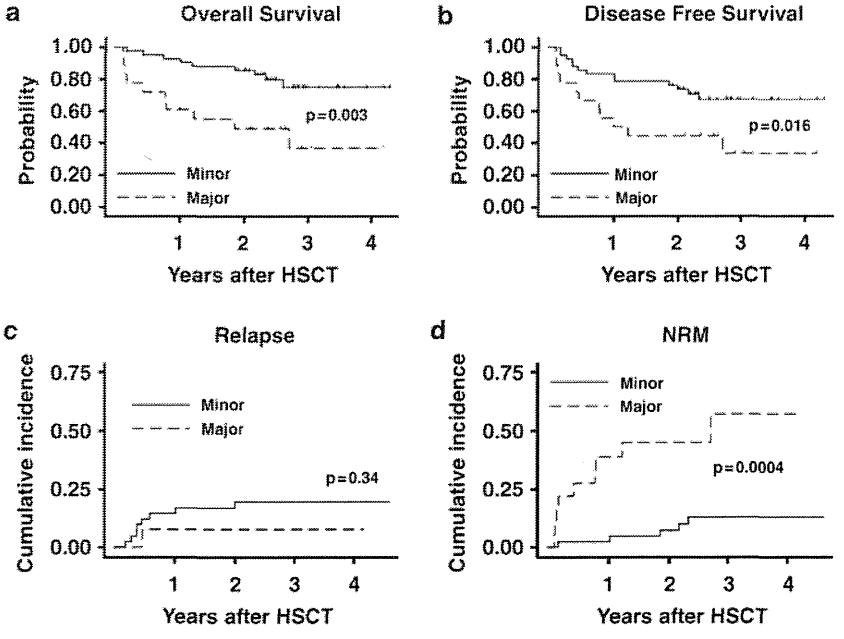

Figure 2. OS and DFS, and cumulative incidence of relapse and NRM related to *BCR–ABL* subtypes in 60 patients with Ph + ALL who underwent allo-HSCT in their first CR following imatinib-based therapy. (**a**) OS, (**b**) DFS, (**c**) cumulative incidence of relapse and (**d**) cumulative incidence of NRM.

the following question would arise: Could pre-existing aberrant gene translocation before allo-HSCT affect the prognosis of patients after transplantation? An inspiring report from Kreil *et al.*[20] verifies a function of $p40^{ABL-BCR}$ fusion protein in the setting of allo-HSCT. They developed a DNA-based deletion screen, and investigated 339 patients with chronic phase CML

Table 4. Patient charateristics according to BCR–ABL subtype in patients with Ph + ALL who received HSCT in their first CR following imatinib-based therapy

	Minor BCR–ABL (%)	*Major BCR–ABL (%)*	P
No. of transplantations	42	18	
Median days from diagnosis to HSCT (range)	149 (84–322)	193 (67–512)	0.090
Conditioning regimen			0.658
Myeloablative	37 (88)	17 (94)	
Reduced intensity	5 (12)	1 (6)	
MRD status before HSCT (3 subjects unknown)			1.000
Positive	13 (32)	5 (29)	
Negative	27 (68)	12 (70)	
HCT-CI (7 subjects unknown)			0.400
0	24 (67)	12 (70)	
1	8 (22)	5 (30)	
2–	4 (11)		
Cause of death			
Leukemia relapse	4 (10)	2 (11)	1.000
Trasnplant related	5 (12)	8 (44)	0.013
Graft failure	1 (2)	4 (22)	
Infection	1 (2)	2 (11)	
cGVHD	1 (2)	1 (5)	
BO	2 (5)		
Others		1 (5)	

Abbreviations: BO, bronchiolitis obliterans; CR, complete remission; cGVHD, chronic graft-versus-host disease; HCT-CI, hematopoietic cell transplantation (HCT)-specific comorbidity index; HSCT, hematopoietic stem cell transplantation; MRD, minimal residual disease; Ph + ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia.

and detected der (9) deletions in 59 (17%) patients. Of these, 21 spanned the *ABL–BCR* junction and 38 were centromeric or telomeric of the breakpoint. Patients with *ABL–BCR* junction-spanning deletions ($p40^{ABL-BCR}$ deficiency) had poorer survival, compared with patients without deletions.[20] More interestingly, this tendency was most distinctive in the setting of allo-HSCT where bone marrow was replaced by normal stem cells from healthy donor.[20] Deletions that did not span the *ABL–BCR* junction were associated with improved survival, compared with patients without deletions. From these, one could speculate that $p40^{ABL-BCR}$ has an important role on the stem-cell re-constitution after allo-HSCT in patients with *BCR–ABL*-positive leukemia, and that, even when the patient's bone marrow was replaced by normal donor stem cells, a deficiency of this protein induced by imatinib-combined chemotherapy could contribute to the relatively high incidence of graft failure (22%) in patients with major *BCR–ABL* transcript as observed in our present study.

Investigation of transplant outcome of Ph + ALL patients who expressed minor *BCR–ABL* transcript and der (9) deletion would be helpful to evaluate clinical relevance of $p96^{ABL-BCR}$. However, to our knowledge, there is no report focusing on the *BCR–ABL* subtypes and der (9) deletions in patients with Ph + ALL. In our present study, three patients who had der (9) deletions were all positive for minor *BCR–ABL* transcript and alive at the last known date of follow-up. Further investigation for clinico-biological effects of not only *BCR–ABL* but also *ABL–BCR* transcripts will be needed to clarify the prognostic relevance of *BCR–ABL* subtypes after allo-HSCT in patients with Ph + ALL.

We categorized two patients with both major and minor *BCR–ABL* transcripts into the major *BCR–ABL* transcript group. Several investigators who studied Ph + ALL with both *BCR–ABL* transcripts have reported that the level of minor *BCR–ABL* transcript was consistently low, such as only one transcript per 100 cells with major *BCR–ABL* transcript.[21] Fujimaki *et al.*[22] studied four patients with Ph + ALL with both transcripts before and after allo-HSCT, and reported that PCR negativity for minor *BCR–ABL* was documented in all cases 1–2 months before PCR negativity for major *BCR–ABL*. Taking these preceding studies into consideration, we believe our categorization of the two patients would be justified.

In the present study, negative MRD before HSCT resulted in significantly lower relapse rate after HSCT (Table 3). Some investigators reported that MRD before HSCT served as a powerful predictor of lower relapse rate and better DFS.[4,23,24] Therefore, prospective monitoring of MRD may potentially identify patients at risk of relapse, although the implications of different transcript levels and increments require validation within each therapeutic context or clinical study.[4] These issues highlight the need for the standardization and harmonization of methodologies used for *BCR–ABL* quantification in Ph + ALL.[4] Employment of highly sensitive methods such as nested PCR or of normalization by total *ABL* transcripts may make clear the predictive value of MRD analysis for the prognosis after HSCT.[25]

To our knowledge, this is the first report on the clinical impact of the *BCR–ABL* subtypes on the outcomes of patients with Ph + ALL after allo-HSCT, analyzing results of a substantial number of patients with a sufficient follow-up period. However, the strength and limitations of our study need to be considered. The strength lies in the relatively large sample size, if not sufficient, and relatively homogenous population, as all patients received a uniform imatinib-combined chemotherapy regimen (JALSG Ph + ALL202)[12] and underwent allo-HSCT in their first CR. These facts gave us a better estimation of the endpoints, and also added statistical power to the analyses. Our limitations are the presence of residual confounding factors, both known and unknown, and insufficient number of patients in each different prognostic factor. Among the known factors, difference in transplantation procedure, including pre-transplant conditioning regimens, should be noted. In this study, conditioning regimens and GVHD prophylaxis were determined by each institution. However, the small number of patients per institution and the changes of the conditioning regimens themselves within the same institution inevitably rendered the analysis on these factors impossible.

We have no comparative clinico-biological data in patients with Ph + ALL transplanted during the pre-imatinib era, and were unable to evaluate whether *BCR–ABL* subtype has a prognostic impact during that time. Further study should be undertaken to evaluate the prognostic value of *BCR–ABL* subtypes both in pre- and post imatinib eras.

The treatment strategy for Ph + ALL in the imatinib era, especially for Ph + ALL with major *BCR–ABL* transcript, should be reconsidered, and additionally, not only allo-HSCT but also second generation tyrosine kinase inhibitors need to be incorporated. Further study would be warranted to determine the clinical impact of *BCR–ABL* transcripts on the outcome of allo-HSCT in this disease.

CONFLICT OF INTEREST

Dr Naoe received research funding and honoraria from Novartis Japan. Dr Ohnishi received research funding from Novartis Japan. Dr Miyazaki received honoraria from Novartis Japan. The remaining authors declare no conflict of interest.

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ORIGINAL ARTICLE

Effect of related donor availability on outcome of AML in the context of related and unrelated hematopoietic cell transplantation

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Although allogeneic hematopoietic cell transplantation (HCT) from a related donor is effective therapy for younger patients with AML, it remains unknown how the availability of a related donor affects the outcome when unrelated HCT is a treatment option for patients without a related donor. To address this issue, we retrospectively analyzed 605 cytogenetically non-favorable AML patients younger than 50 years for whom a related donor search was performed during first CR (CR1). The 4-year OS was 62% in 253 patients with a related donor and 59% in 352 patients without a related donor ($P = 0.534$). Allogeneic HCT was performed during CR1 in 62% and 41% of patients with and without a related donor, respectively. Among patients transplanted in CR1, the cumulative incidence of non-relapse mortality was significantly higher in patients without a related donor ($P = 0.022$), but there was no difference in post-transplant OS between the groups ($P = 0.262$). These findings show the usefulness of unrelated HCT in younger patients with cytogenetically non-favorable AML who do not have a related donor. The extensive use of unrelated HCT for such patients may minimize the potential disadvantage of lacking a related donor.

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Keywords: AML; allogeneic hematopoietic cell transplantation; donor; related transplantation; unrelated transplantation; first CR

INTRODUCTION

Owing to the strong anti-leukemic effect of pre-transplant conditioning therapy in combination with the post-transplant GVL effect, allogeneic hematopoietic cell transplantation (HCT) is currently the most powerful method for preventing relapse of AML.[1] However, the efficacy of allogeneic HCT is compromised by a high risk of treatment-related mortality, which raises the question of whether allogeneic HCT is truly beneficial for AML patients who are in their first CR (CR1). Historically, this question has been investigated in prospective studies that used biologic assignment according to donor availability, in which patients with an HLA-identical sibling donor were assigned to allogeneic HCT, whereas those without an HLA-identical sibling donor were assigned to chemotherapy and/or autologous HCT.[2–8] If we combine the results from those studies, we find that allogeneic HCT during CR1 confers a survival advantage in patients with cytogenetically intermediate and unfavorable risk.[8–10] However, such 'donor vs no-donor' studies do not provide an accurate picture of clinical practice, because an HLA-identical sibling is not the only donor source and a substantial proportion of patients without a related donor receive allogeneic HCT from an unrelated donor.

To examine how related donor availability affects the outcome of AML in a situation where unrelated HCT is a treatment option for patients without a related donor, we retrospectively analyzed cytogenetically non-favorable AML patients under the age of 50 years for whom a related donor search was conducted during CR1. The main objectives of this study were to assess the difference in survival according to related donor availability in terms of (1) overall outcome, (2) outcome after allogeneic HCT in CR1 (that is, comparison between related and unrelated HCT) and (3) outcome after first relapse following chemotherapy. We also looked at how unrelated HCT was incorporated into the treatment strategy in our patient cohort.

PATIENTS AND METHODS

Patients

Adults with AML who had achieved CR1 were retrospectively registered in a Japanese nationwide AML database, which formed the basis of this study. Seventy institutions contributed patients to the database. Patients were eligible if they were younger than 50 years, were diagnosed with AML from 1999 to 2006 according to the World Health Organization (WHO) classification,[11] had achieved CR with one or two courses of chemotherapy, and had a related donor search performed during CR1. We excluded patients with acute promyelocytic leukemia and core-binding factor AML, as well as those whose pre-treatment cytogenetic results were not available. Patients who underwent haploidentical HCT were also excluded. Overall, 605 patients fulfilled these criteria, and thus were subjected to

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subsequent analyses. Information was collected and compiled with regard to patient-related factors (that is, age and sex), disease-related factors (that is, cytogenetics, WBC count and dysplasia in morphology), number of induction courses, related donor availability, and clinical outcome. For patients who underwent allogeneic HCT, complementary information on HCT (that is, interval from CR1 to HCT, disease status at time of HCT, conditioning regimen and donor source) was also collected. Patients were considered to have a related donor if HLA typing identified a matched or one Ag-mismatched family donor. Unrelated donor selection was based on matching at the level of resolution available at the time of transplantation. This study was approved by the Institutional Review Board at the National Cancer Center Hospital.

Statistical analysis

Distributions of patient characteristics between groups were compared by using the χ^2 test for categorical variables and the Wilcoxon rank-sum test for continuous variables. The probabilities of OS and relapse-free survival (RFS) were estimated by the Kaplan–Meier method, with differences between groups qualified by the log-rank test. Relapse and non-relapse mortality (NRM) were considered as competing risk events for each other. The probabilities of relapse and NRM were estimated by the cumulative incidence functions, and differences between groups were qualified by the Gray test. The Cox proportional hazards regression model was used for univariate and multivariate analyses, and a hazard ratio (HR) was calculated in conjunction with a 95% confidence interval (CI). All statistical analyses were performed with SPSS software version 11.0.1 (SPSS, Chicago, IL, USA) and R software version 2.13.0 (The R Foundation for Statistical Computing).

RESULTS

Patient characteristics

Of the 605 patients eligible for analysis, a related donor was found for 253 patients (42%) during CR1. There were no significant differences between the groups in the distribution of baseline characteristics, with the exception of WBC count (Table 1). Figure 1 shows the patient flow with respect to related donor availability, allogeneic HCT in CR1 and relapse. Among the 253 patients with a related donor, 157 (62%) underwent allogeneic HCT in CR1 (156 from a related donor and 1 from an unrelated BM donor). Of the 352 patients without a related donor, allogeneic HCT was performed during CR1 in 146 patients (41%), of whom 109 and 37 received unrelated BMT and umbilical cord blood (UCB) transplantation, respectively. In all, 96 patients with a related donor and 206 patients without a related donor did not receive allogeneic HCT during CR1. Among them, 25 (26%) and 49 (24%) patients experienced early relapse within 6 months after achievement of CR1. Autologous HCT was performed during CR1 in 5 and 14 patients with and without a related donor, respectively.

Characteristics of patients who underwent allogeneic HCT in CR1

The characteristics of patients who underwent allogeneic HCT in CR1 are summarized according to related donor availability in Table 2. The two groups were well balanced in terms of baseline characteristics. However, patients without a related donor were more likely to receive two courses of induction therapy instead of one course ($P = 0.023$). The interval from CR1 to transplantation differed significantly between the groups (Figure 2), with a median interval of 3.7 months for patients with a related donor vs 5.9 months for patients without a related donor ($P < 0.001$). Myeloablative conditioning was used in 89% and 88% of patients with and without a related donor, respectively.

Outcome after CR1 according to related donor availability

The median follow-up of surviving patients was 4.4 years (range, 0.1–9.7), and the 4-year OS was 60% for the entire population. Figure 3 shows Kaplan–Meier survival estimates for patients with and without a related donor. The 4-year OS was 62% in patients with a related donor and 59% in patients without a related donor, with no significant difference detected ($P = 0.534$). Similar results were obtained when the analysis was restricted to patients with unfavorable cytogenetic risk (51% vs 44% at 4 years, $P = 0.213$) or those with intermediate cytogenetic risk (67% vs 67% at 4 years, $P = 0.744$). In the multivariate analysis, cytogenetics and number of induction courses were identified as factors that were significantly associated with OS, whereas related donor availability had no significant impact (Table 3).

Table 1. Patient characteristics

	Related donor + N = *253*	*Related donor −* N = *352*	P-*value*
Age, years			0.547
Median	34	35	
Range	16–49	16–49	
Sex			0.232
Male	127 (50%)	194 (55%)	
Female	126 (50%)	158 (45%)	
Cytogenetic risk by SWOG			0.74
Intermediate	153 (60%)	211 (60%)	
Unfavorable	72 (28%)	94 (27%)	
Unknown	28 (11%)	47 (13%)	
WBC count, × 10^9/L			0.049
Median	13.8	18	
Range	0.6–794.0	0.5–410.7	
Dysplasia			0.9
No	204 (81%)	285 (81%)	
Yes	49 (19%)	67 (19%)	
No. of induction courses			0.186
1 Course	196 (77%)	256 (73%)	
2 Courses	57 (23%)	96 (27%)	
Allogeneic HCT			<0.001
CR1	157 (62%)	146 (41%)	
CR2	27 (11%)	44 (13%)	
Other disease phases	38 (15%)	63 (18%)	
Not performed	31 (12%)	99 (28%)	

Abbreviations: CR1 = first CR; CR2 = second CR; SWOG = Southwest Oncology Group.

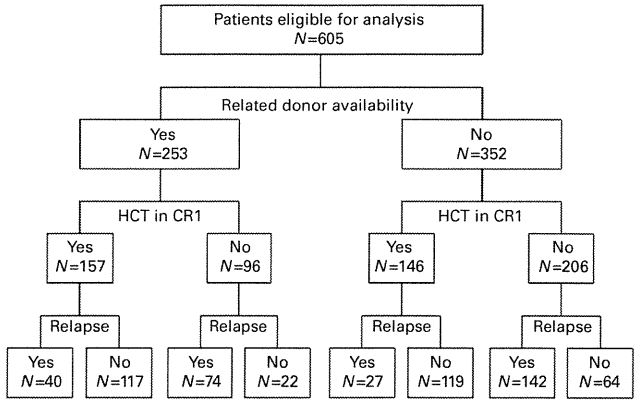

Figure 1. Flow diagram of patients.

Table 2. Characteristics of patients who underwent allogeneic HCT in CR1

	Related donor+ N = *157*	*Related donor−* N = *146*	P-*value*
Age, years			0.441
Median	34	35	
Range	16–49	16–49	
Sex			0.252
Male	80 (51%)	84 (58%)	
Female	77 (49%)	62 (42%)	
Cytogenetic risk by SWOG			0.178
Intermediate	97 (62%)	78 (53%)	
Unfavorable	40 (25%)	47 (32%)	
Unknown	20 (13%)	21 (14%)	
WBC count, × 10^9/L			0.644
Median	12.7	11.5	
Range	0.9–794.0	0.6–410.7	
Dysplasia			0.729
No	118 (75%)	112 (77%)	
Yes	39 (25%)	34 (23%)	
No. of induction courses			0.023
1 Course	115 (73%)	89 (61%)	
2 Courses	42 (27%)	57 (39%)	
Interval from CR1 to HCT, days			<0.001
Median	113	178	
Range	0–620	14–770	
Type of donor			<0.001
Related	156 (99%)	0 (0%)	
Unrelated, BM	1 (1%)	109 (75%)	
Unrelated, cord blood	0 (0%)	37 (25%)	
Type of conditioning			0.969
Myeloablative	140 (89%)	128 (88%)	
Reduced-intensity	14 (9%)	13 (9%)	
Not specified	3 (2%)	5 (3%)	

Abbreviations: CR1 = first CR; HCT = hematopoietic cell transplantation; SWOG = Southwest Oncology Group.

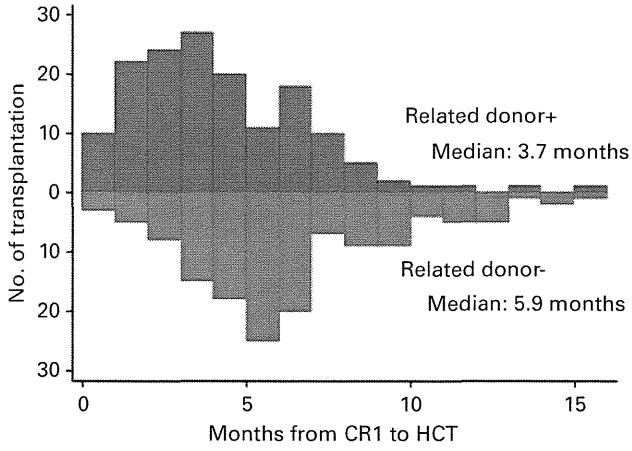

Figure 2. Interval from CR1 to HCT in patients who underwent allogeneic HCT during CR1. Patients with (Related donor +, $N = 157$) and without a related donor (Related donor −, $N = 146$) are shown separately. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

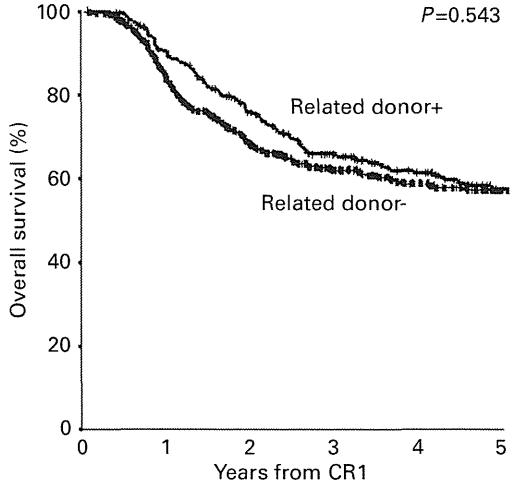

Figure 3. Kaplan–Meier curves for OS after CR1 according to related donor availability. All patients with (Related donor +, $N = 253$) and without a related donor (Related donor −, $N = 352$) are compared.

Outcome after allogeneic HCT in CR1 according to related donor availability

Figure 4 compares post-transplant OS between patients with and without a related donor who underwent allogeneic HCT during CR1. There was no difference in OS between the groups: the 4-year OS was 65% in patients with a related donor and 61% in patients without a related donor ($P = 0.262$). The cumulative incidence of NRM in patients with a related donor was significantly lower than that in patients without a related donor (13% vs 21% at 4 years, $P = 0.022$). In terms of relapse, patients with a related donor appeared to show a higher incidence, but the difference was not statistically significant (26% vs 21% at 4 years, $P = 0.292$). OS with unrelated BMT was superior to that with UCB transplantation (66% vs 48% at 4 years, $P = 0.044$): the former was equivalent to the result with related HCT ($P = 0.897$), whereas the latter was worse ($P = 0.003$). Related HCT from a matched ($N = 140$) and one Ag-mismatched donor ($N = 16$) showed no difference in OS (66% vs 56% at 4 years, $P = 0.304$).

Effect of allogeneic HCT during CR1 in patients with or without a related donor

To examine how allogeneic HCT in CR1 impacted RFS and OS, we performed separate multivariate analysis for patients with and without a related donor. In this analysis, HCT was considered as a time-dependent covariate, and adjustments were made for all of the variables listed in Table 3 except for related donor availability. In patients with a related donor, allogeneic HCT in CR1 was associated with superior RFS (HR, 0.28; 95% CI, 0.19–0.41) and OS (HR, 0.65; 95% CI, 0.43–0.98). In patients without a related donor, allogeneic HCT in CR1 had favorable effect on RFS (HR, 0.58; 95% CI, 0.41–0.82) and OS (HR, 0.82; 95% CI, 0.56–1.19), although the effect on OS did not reach statistical significance.

Outcome after first relapse following chemotherapy according to related donor availability

Among patients who did not undergo allogeneic HCT in CR1, 74 patients with a related donor and 142 patients without a related

Table 3. Factors associated with OS

	Univariate analysis			*Multivariate analysis*		
	HR	*(95% CI)*	P-*value*	*HR*	*(95% CI)*	P-*value*
Related donor availability						
Yes	1		—	1		—
No	1.09	(0.84–1.40)	0.534	1.08	(0.83–1.39)	0.576
Age						
As a numerical variable (per 1 year)	1	(0.99–1.01)	0.799	1	(0.99–1.01)	0.926
Sex						
Male	1		—	1		—
Female	0.98	(0.76–1.27)	0.899	1	(0.78–1.30)	0.979
Cytogenetic risk by SWOG						
Intermediate	1		—	1		—
Unfavorable	1.92	(1.45–2.54)	<0.001	2.00	(1.51–2.65)	<0.001
Unknown	1.72	(1.18–2.50)	0.005	1.52	(1.04–2.22)	0.031
WBC count						
As a numerical variable (per 10×10^9/L)	1.02	(1.00–1.03)	0.035	1.01	(1.00–1.03)	0.065
Dysplasia						
No	1		—	1		—
Yes	0.7	(0.49–1.00)	0.052	0.71	(0.50–1.02)	0.061
No. of induction courses						
1 Course	1		—	1		—
2 Courses	2.44	(1.88–3.17)	<0.001	2.46	(1.88–3.21)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; SWOG = Southwest Oncology Group.

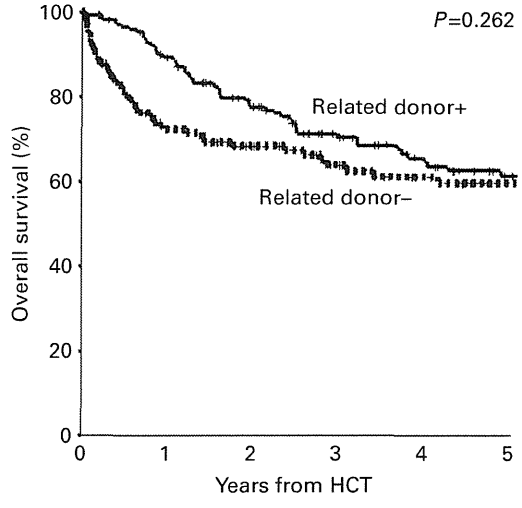

Figure 4. Kaplan–Meier curves for OS after allogeneic HCT in CR1 according to related donor availability. Patients with (Related donor+, $N = 157$) and without a related donor (Related donor−, $N = 146$) who underwent allogeneic HCT in CR1 are compared.

donor experienced relapse (Figure 1). After relapse, 65 (88%) patients with a related donor received allogeneic HCT (62 from a related donor, 2 from an unrelated BM donor and 1 from UCB), as did 107 (75%) patients without a related donor (63 from an unrelated BM donor, 42 from UCB and 1 from a related donor who had not been included in the initial related donor search; information was missing for 1 patient). In all, 27 patients with a related donor and 44 patients without a related donor received allogeneic HCT during CR2. For patients who experienced relapse without having received allogeneic HCT in CR1, the 4-year OS after relapse was 33% in patients with a related donor and 33% in patients without a related donor ($P = 0.245$).

DISCUSSION

The outcome of unrelated HCT has recently improved primarily due to the introduction of high-resolution HLA-typing technology and improvements in supportive care. In addition, the growth of unrelated donor registries as well as the increased use of UCB grafts has increased the chance of finding an unrelated donor.[1] These advances have made unrelated HCT a more feasible option for patients who lack a related donor. As our analyses were based on a nationwide multicenter survey, the finding that 41% of patients without a related donor received unrelated HCT during CR1 reflects the widespread use of unrelated HCT in Japan. On the other hand, in patients with a related donor, the proportion of patients who underwent allogeneic HCT during CR1 reached 62%. This value was comparable to or only slightly lower than the HCT compliance rates reported in previous donor vs no-donor studies, where allogeneic HCT was offered to all patients with a related donor as per the study protocol.[2–8] These findings show that allogeneic HCT, from both related and unrelated donors, was actively incorporated into the treatment strategy in our patient population.

When we take into account that patients with core-binding factor AML were excluded from our study, the 60% 4-year OS for the entire cohort appears quite favorable. Recently, the Japan Adult Leukemia Study Group reported results from a prospective study (designated AML201) for newly diagnosed AML patients, in which standard-dose and high-dose cytarabine (AraC)-based regimens were compared for post-remission therapy.[12] In that study, for patients younger than 50 years, the 5-year OS was 66%

with standard-dose AraC consolidation and 62% with high-dose AraC consolidation. As the AML201 study included patients with core-binding factor AML (28% of the total population), and the patients in that study were selected according to the pre-defined inclusion and exclusion criteria, it is remarkable that a comparable survival rate was achieved in our patients. The active use of allogeneic HCT not only in patients with a related donor but also in those without a related donor likely contributed to the favorable overall outcome of our patients.

In contrast to the results of meta-analysis studies of the prospective donor vs no-donor comparison,[8–10] our patients with and without a related donor had comparable OS. Similar results were obtained if the outcome was compared in terms of RFS (data not shown). The most likely explanations for this result is that up to 41% of our patients without a related donor proceeded to unrelated HCT during CR1, and that OS after allogeneic HCT in CR1 did not differ between patients with and without a related donor. NRM is a major obstacle to the success of unrelated HCT. Early studies showed less satisfactory results with unrelated HCT because of a high incidence of NRM.[13,14] However, according to more recent data, comparable outcomes have been reported for related and unrelated HCT in AML patients.[15–18] In our study, the cumulative incidence of NRM in patients undergoing unrelated HCT was significantly higher than that in those undergoing related HCT (21% vs 13%, $P = 0.022$), but the NRM rate of 21% with unrelated HCT appears to be within the acceptable range. The benefits of unrelated HCT may be increased by reducing NRM with the aid of stricter matching between donor and patient, increasing the use of reduced-intensity conditioning, and applying better supportive care. Recently, several groups have conducted prospective donor vs no-donor studies for AML patients with high-risk features by expanding the type of donor to include unrelated donors.[19–21] Notably, despite a limited number of patients in each study, they showed significantly superior OS in patients with a donor, as well as comparable OS in patients undergoing related and unrelated HCT.[19–21] These prospective studies also support the usefulness of unrelated HCT in younger AML patients with non-favorable cytogenetics. Although our multivariate analysis showed that the degree to which allogeneic HCT had favorably affected outcome was less marked in patients without a related donor compared with those with a related donor, unrelated HCT could be considered a reasonable treatment option if a related donor is not available.

When our data are interpreted, it should be remembered that this study was an observational study, not an interventional study. The decision of whether or not to proceed to allogeneic HCT could be confounded by multiple factors, and early relapse, for example, did not seem to be a main cause for not having undergone allogeneic HCT during CR1 in our study. Adjusting for known confounding factors by using a multivariate analysis cannot guarantee that biases are removed. Thus, the results presented here need to be interpreted cautiously. Although we acknowledge such a limitation, our data showed that related donor availability did not significantly affect OS in younger patients with cytogenetically non-favorable AML. We consider this was because 41% of the patients without a related donor underwent unrelated HCT during CR1, and the outcome after transplantation was comparable between related and unrelated HCT. These results show the usefulness of unrelated HCT in this patient population when they do not have a related donor. The extensive use of unrelated HCT for such patients may minimize the potential disadvantage of lacking a related donor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: MY designed the study, interpreted data, and wrote the manuscript; SK prepared the data file, performed the analysis and interpreted data; TY was primarily responsible for the study design, data analysis and interpretation of the data; NU, SM, HK, KU, TK, MW, KN, SY, Y Nawa, JT, HT, Y Nakamura, SF and FK obtained the patients' data and interpreted data; NE designed the study and interpreted data; IM reviewed the cytogenetic reports and interpreted data; and TF interpreted data and helped to write the manuscript.

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bjh research paper

Outcome after first relapse in adult patients with Philadelphia chromosome-negative acute lymphoblastic leukaemia

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Summary

To analyse the outcome of adult patients who developed a first relapse of acute lymphoblastic leukaemia (ALL), we collected the clinical data of 332 patients with Philadelphia-chromosome (Ph) negative ALL, aged 16–65 years, who relapsed after first complete remission (CR1) between 1998 and 2008 in 69 institutions all over Japan, including 58 patients who relapsed after allogeneic haematopoietic stem cell transplantation (Allo-HSCT) in CR1. The overall survival (OS) was 43·4% at 1 year, and 16·3% at 5 years from relapse in patients who received chemotherapy alone in CR1. Among patients who relapsed after chemotherapy alone in CR1, 123 (52·5%) achieved a second remission (CR2) following salvage chemotherapy, of whom 62 subsequently underwent Allo-HSCT during CR2. Allo-HSCT in CR2 was significantly associated with better OS. Moreover, the type of salvage chemotherapy influenced OS from relapse. A doxorubicin, vincristine, and predonisone-based (AdVP-type) regimen was related to better OS in patients with longer CR1 (more than 1 year), but was related to worse OS in patients with shorter CR1. In conclusion, the prognosis of patients with relapsed Ph-negative ALL is poor. Allo-HSCT after a first relapse could improve the prognosis. Selection of the optimal salvage chemotherapy might depend on the duration of CR1.

Keywords: acute lymphoblastic leukaemia, first relapse, second remission, salvage chemotherapy, allogeneic haematopoietic stem cell transplantation.

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The complete remission (CR) rate of adult patients with acute lymphoblastic leukaemia (ALL) has improved to about 90% with modern intensive chemotherapy. However, many patients eventually relapse, and the long-term leukaemia-free survival rate is only 30–40% (Litzow, 2009). Many relapsed patients receive various salvage therapies after the first relapse, and several studies have reported that 38–56% of relapsed patients can achieve second remission (CR2; Giona *et al*, 1997; Thomas *et al*, 1999; Camera *et al*, 2004; Tavernier *et al*, 2007; Cornelissen *et al*, 2009). While allogeneic haematopoietic stem cell transplantation (Allo-HSCT) in CR2 is considered to be the only curative strategy, early relapse and/or organ dysfunction after salvage chemotherapy and the lack of a suitable donor often prevent Allo-HSCT at this stage. Therefore, the prognosis of adult patients with relapsed ALL is extremely poor (Fielding *et al*, 2007; Tavernier *et al*, 2007; Oriol *et al*, 2010; Gokbuget *et al*, 2012). We collected clinical data after the first relapse in adult patients with Philadelphia chromosome (Ph)-negative ALL who were treated in institutions all over Japan, and performed a retrospective analysis to clarify the prognosis and prognostic factors for the outcome in relapsed patients. Patients with Ph-positive ALL were not included in our analysis because the outcome of treatment in these patients has improved dramatically since tyrosine kinase inhibitors became available (Ottmann & Pfeifer, 2009).

Methods

Data source

We retrospectively collected clinical data of patients with Ph-negative ALL, aged 16–65 years, who relapsed after achieving first remission (CR1) between 1998 and 2008. Patients with lymphoblastic lymphoma were not included. Patients had received either chemotherapy alone, autologous stem cell transplantation (Auto-HSCT), or Allo-HSCT in CR1. Patients who did not achieve remission with chemotherapy but eventually achieved remission after HSCT were excluded. CR was defined based on haematological findings, and not on molecular findings. The data collected included clinical information about the disease at both diagnosis and relapse, the content of therapies both at diagnosis and after relapse, and the clinical course. This study was approved by the Institutional Review Board of Saitama Medical Centre, Jichi Medical University.

Statistical considerations

Differences between groups were examined using the Fisher's exact test for categorical variables and the Mann-Whitney U-test for continuous variables. Overall survival (OS) was calculated using the Kaplan-Meier method. To evaluate the influence of factors for OS, the log-rank test and proportional-hazards modelling were used for univariate and multivariate analyses, respectively. In a univariate analysis, the impact of Allo-HSCT in CR2 was assessed by a landmark analysis that was limited to patients who achieved CR2 and survived without relapse for at least 68 d, which was the median duration between the achievement of CR2 and the performance of Allo-HSCT in CR2. A post-hoc multicomparison test using the Holm method was performed for comparisons among three groups.

For the multivariate analysis, we included all covariates at first, and used the backward stepwise selection of covariates. Finally, P values of less than 0·05 were considered statistically significant. Potential confounding factors at diagnosis that were considered in the analysis were sex, phenotype of ALL, white blood cell (WBC) count, karyotype (patients with t(4;11) or complex karyotype (5 or more chromosomal abnormalities) were considered high-risk, and were compared to those with other karyotypes), presence of central nervous system (CNS) invasion of ALL, intensity of chemotherapy, and duration between the diagnosis and the achievement of CR1. Moderate-intensity regimens at diagnosis were defined as those that included both high-dose cytarabine and high-dose methotrexate, and the other regimens were regarded as conventional-intensity. In addition, potential confounding factors at relapse that were considered in the analysis were relapse year, age, duration of CR1, sites of relapse, WBC count, and additional chromosomal abnormalities. Allo-HSCT in CR2 was also included as a time-dependent covariate in the multivariate analysis.

We specifically evaluated the influence of salvage chemotherapy after relapse on OS. Salvage chemotherapy regimens were categorized into three types. The first was combination chemotherapy based on conventional doses of doxorubicin, vincristine, and predonisone (AdVP-type). The second type was fractionated cyclophosphamide,

vincristine, doxorubicin, and dexamethasone alternating with high doses of methotrexate and cytarabine (hyperC-VAD/MA; Kantarjian *et al*, 2000), and the third type included high-dose cytarabine (Kantarjian *et al*, 1986), the combination of mitoxantrone, etoposide, and cytarabine (MEC; Amadori *et al*, 1991), and the combination of high-dose cytarabine and mitoxantrone (HAM; Lejeune *et al*, 1990). Potential confounding and selection biases for these three kinds of salvage regimens were considered through the Fisher's exact test.

All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University; Kanda, 2012),

Table I. Patient characteristics.

	All patients (n = 332)	Relapse after CTx alone (n = 270)	Relapse after Allo-HSCT (n = 58)	P value*
Patient characteristics at diagnosis				
Age, years; median (range)	34 (15–65)	37 (15–65)	28 (16–58)	0·006
Sex				
Male	165 (50%)	132 (49%)	30 (52%)	0·773
Female	167 (50%)	138 (51%)	28 (48%	
Phenotype				
T	65 (20%)	47 (17%)	17 (29%)	0·038
B	253 (76%)	213 (79%)	37 (64%)	
Other	6 (2%)	4 (1%)	2 (3%)	
WBC, $\times 10^9$/l; median (range)	11·1 (0·6–759·7)	11·0 (0·6–759·7)	14·4 (0·6–537·9)	0·264
Karyotype				
normal	162 (49%)	138 (51%)	20 (34%)	0·025
t(4;11)	10 (3%)	10 (4%)	0 (0%)	
complex	50 (15%)	36 (13%)	14 (24%)	
others	84 (25%)	66 (25%)	18 (31%)	
CNS invasion				
–	312 (94%)	255 (94%)	53 (91%)	0·238
+	12 (4%)	8 (3%)	4 (7%)	
CTx intensity†				
Conventional	184 (55%)	149 (55%)	32 (55%)	1·0
Moderate	142 (43%)	117 (43%)	25 (43%	
Days from diagnosis to CR1; median (range)	38 (14–337)	37 (14–337)	42 (21–143)	0·081
Patient characteristics at relapse				
Relapse year				
Upto end 2003	152 (46%)	127 (47%)	21 (36%)	0·147
2004 onwards	180 (54%)	143 (53%)	37 (64%)	
Follow-up after relapse, days; median (range)	1344 (12–3689)	1381 (12–3689)	1001 (726–2799)	0·540
Duration of CR1				
Median (range) (d)	290 (15–7162)	246 (15–7162)	465 (33–2185)	<0·001
<1 year	193 (58%)	171 (63%)	22 (38%)	
≥ 1 year	135 (41%)	96 (36%)	35 (60%)	
Sites of relapse				
BM included	288 (87%)	237 (88%)	47 (81%)	<0·001
CNS alone	29 (9%)	28 (10%)	1 (2%)	
others	13 (4%)	4 (1%)	9 (16%)	
Age, years; median (range)	35 (16–65)	37 (16–65)	29 (18–59)	0·011
WBC, $\times$ 10^9/l; median (range)	4·5 (0·6–331·6)	4·4 (0·6–331·6)	5·1 (1·2–137·3)	0·168
Additional chromosomal abnormality				
–	231 (70%)	198 (73%)	29 (50%)	0·01
+	52 (16%)	37 (14%)	15 (26%)	
Patients that achieved CR2, n	163 (53·6%)	136 (54·0%)	26 (54·2%)	0·753

*P value was calculated based on the comparison of patients who relapsed after CTx alone in CR1 to those who relapsed after Allo-HSCT in CR1. Differences between these two groups were examined using the Fisher's exact test for categorical variables and the Mann–Whitney U-test for continuous variables.

†Moderate-intensity regimens included both high-dose cytarabine and high-dose methotrexate.

CTx, chemotherapy; Allo-HSCT, allogeneic haematopoietic stem cell transplantation; WBC, white blood cell; CNS, central nervous system; CR, complete remission; BM, bone marrow.

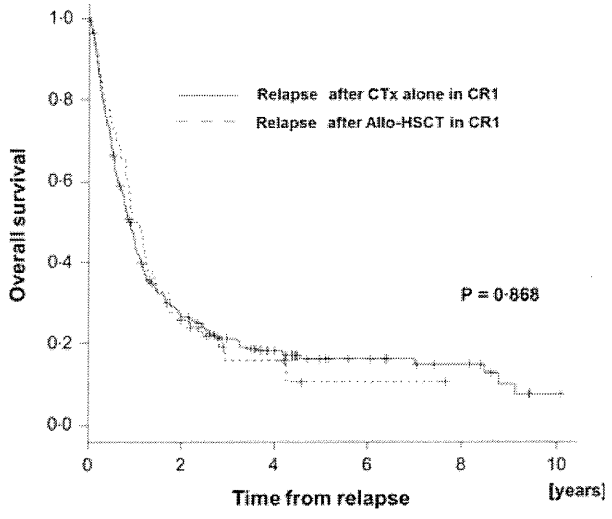

Fig 1. Overall survival from relapse. Overall survival (OS) from relapse in relapsed patients after chemotherapy (CTx) alone in first complete remission (CR1) and in those after allogeneic haematopoietic stem cell transplantation (Allo-HSCT) in CR1. The OS rate was not significantly different between these two groups.

which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0).

Results

Patient characteristics

A total of 332 patients, treated in 69 institutions all over Japan, were included in this study. The characteristics of the patients are summarized in Table I. Their median age at relapse was 35 years, and 165 patients were male. The median duration of CR1 was 290 d (range 15–7162 d), and the median follow-up time after relapse was 1344 d (range 12–3689 d). Two-hundred and seventy patients relapsed after chemotherapy alone in CR1, and 58 and 4 patients relapsed after Allo- and Auto-HSCT in CR1, respectively. When the patients who relapsed after chemotherapy alone in CR1 were compared to those who relapsed after Allo-HSCT in CR1, the distribution of the phenotype and karyotypes of leukaemic cells were significantly different. In addition, older age at relapse, shorter duration of CR1, and lower rate of the appearance of additional chromosomal abnormalities at relapse were observed in patients who relapsed after chemotherapy alone in CR1.

Overall survival (OS) from relapse in the 270 patients who received chemotherapy alone in CR1 was 43·4% at 1 year and 16·3% at 5 years, while OS from relapse in the 58 patients who received Allo-HSCT in CR1 was 50·0% at 1 year and 10·6% at 5 years. The OS rate was not significantly different between these two groups (hazard ratio (HR): 0·97, 95% confidence interval (CI): 0·64–1·29, $P = 0·868$; Fig 1).

Outcome of relapsed patients after chemotherapy alone in CR1

Among patients who relapsed after chemotherapy alone in CR1, 43% had received moderate-intensity regimens at diagnosis, such as hyperCVAD/MA (Kantarjian *et al*, 2000) and regimens used in the Japan Adult Leukaemia Study Group (JALSG) ALL201 studies (C000000063 for older patients and C000000064 for younger patients; Sakura *et al*, 2012). The remaining patients had received conventional-intensity regimens, which were mainly used in JALSG ALL93 (Takeuchi *et al*, 2002) and ALL97 (Jinnai *et al*, 2010) studies.

Among the 270 patients who relapsed after chemotherapy alone in CR1, 234 received salvage chemotherapy, and 123 of these 234 (52·5%) achieved a second complete remission (CR2; Fig 2). Sixty-two of these 123 patients underwent Allo-HSCT in CR2, 16 patients from a human leucocyte antigen (HLA)-matched related donor, 6 from an HLA-mismatched related donor, and 30 from an unrelated donor. The remaining 10 received unrelated cord blood. Fifty-five of them received myeloablative conditioning. The median duration between the achievement of CR2 with salvage chemotherapy and Allo-HSCT in CR2 was 68 d (range 10–276 d). OS from CR2 was significantly better in patients who under-

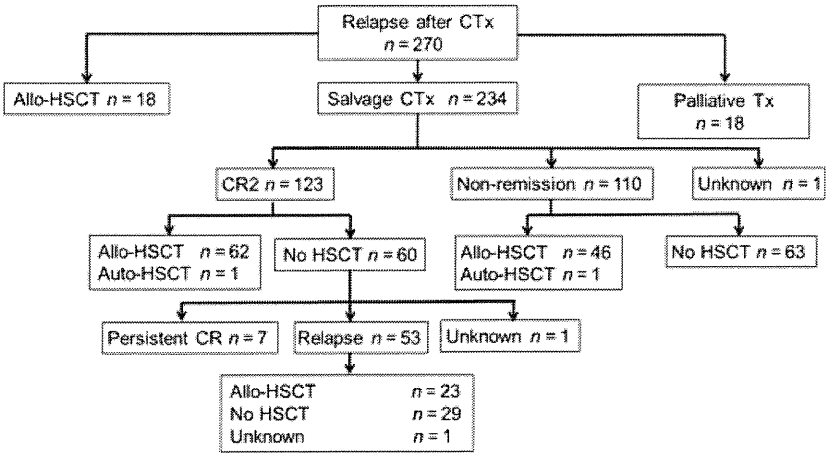

Fig 2. Clinical course of relapsed patients after chemotherapy alone in the first complete remission. Among the 270 patients who relapsed after chemotherapy (CTx) alone in first complete remission (CR1), 234 received salvage CTx, and 123 of these 234 patients (52·5%) achieved a second CR (CR2). Eighteen patients underwent allogeneic haematopoietic stem cell transplantation (Allo-HSCT) directly after relapse without salvage CTx. Tx, treatment; Auto-HSCT, autologous haematopoietic stem cell transplantation

went Allo-HSCT in CR2 than in those who did not (73·7% vs. 50·0% at 1 year and 43·9% vs. 11·0% at 5 years, respectively; HR: 0·43, 95% CI: 0·26–0·72, $P = 0·001$) by a landmark analysis that was limited to patients who were alive without disease at 68 d after they had achieved CR2 (Fig 3). The results of a Mantel-Byar analysis that evaluated the influence of Allo-HSCT in CR2 were similar to that of a landmark analysis using the log-rank test (HR: 0·49, 95% CI: 0·31–0·77, $P = 0·002$). In a multivariate analysis using the backward stepwise selection of covariates, younger age at relapse (younger than 36 years), lower WBC count at relapse (less than 10×10^9/l), and Allo-HSCT in CR2 treated as a time-dependent covariate were associated with better OS among patients who achieved CR2 following salvage chemotherapy (Table II).

We further analysed the effect of each salvage regimen that patients received after the first relapse. Patients who received one of the three types of salvage regimen below were included in this analysis. Those who had isolated CNS relapse were excluded. Seventy-five patients received the AdVP-type salvage regimen, and 58 received the hyperCVAD/MA regimen (Kantarjian *et al*, 2000). The other 46 patients received a high-dose cytarabine (Kantarjian *et al*, 1986; $n = 22$), MEC (Amadori *et al*, 1991; $n = 14$), or HAM (Lejeune *et al*, 1990; $n = 10$) regimen. The type of salvage regimen did not significantly influence OS from relapse (Fig 4A). In the univariate analyses, a longer duration of CR1 (more than 1 year; $P = 0·003$), the intensity of chemotherapy at diagnosis ($P < 0·001$), and the relapse year (before 31 December 2003 or after 1 January 2004; $P = 0·008$) were significantly associated with the selected salvage regimen. Therefore, the patients included in this analysis were stratified into two groups according to the duration of CR1, intensity of chemotherapy at diagnosis, and relapse year, respectively. The duration of CR1 was less than 1 year in 96 patients and longer than 1 year in 71 patients. In patients who had a shorter duration of CR1, the type of salvage regimen influenced OS ($P = 0·002$; Fig 4B). In the post-hoc test, patients who received the AdVP-type regimen had worse OS, compared to those who received the hyperCVAD/MA regimen ($P = 0·040$) or other regimens ($P < 0·001$). The type of salvage regimen also influenced OS in patients who had a longer duration of CR1 ($P = 0·048$; Fig 4C). In contrast, the post-hoc test indicated that patients who received the AdVP-type regimen were associated with better OS with borderline significance, compared to those who received the hyperCVAD/MA regimen ($P = 0·066$). When patients were stratified into two groups according to the intensity of chemotherapy at diagnosis or the relapse year, the type of salvage regimen did not influence OS ($P = 0·733$ and 0·843, in patients who received conventional- and moderate-intensity chemotherapy at diagnosis, respectively, and $P = 0·131$ and 0·892, in patients who relapsed before 31 December 2003 and after 1 January 2004, respectively).

Table II. Prognostic factors in patients who relapsed after chemotherapy alone in first complete remission (CR1) and achieved second CR (CR2) following salvage chemotherapy.

	Multivariate analysis	
	Hazard ratio (95% CI)	*P* value
Allo-HSCT in CR2*		
+	0·49 (0·31–0·79)	0·003
−		
Age at relapse		
>35 years	1·83 (1·18–2·82)	0·007
≤35 years		
WBC count at relapse		
≥10 × 10^9/l	2·87 (1·67–4·95)	<0·001
<10 × 10^9/l		

*Allo-HSCT in CR2 was treated as a time-dependent covariate.
CI, confidence interval; allo-HSCT, allogeneic haematopoietic stem cell transplantation; CR, complete remission; WBC, white blood cell.

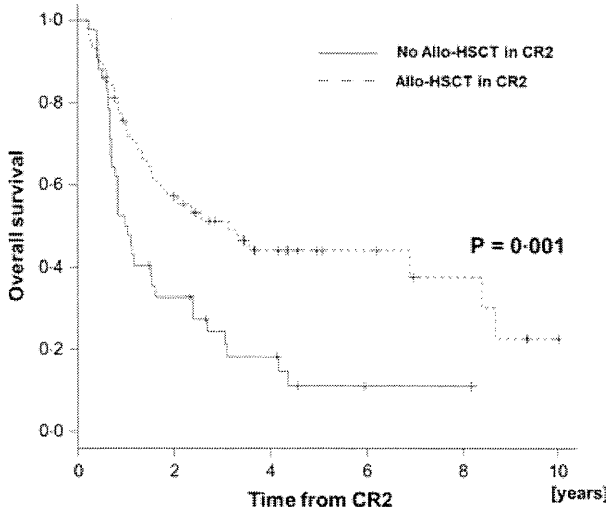

Fig 3. Overall survival from the second complete remission (CR) in patients who relapsed after chemotherapy alone in the first CR. Among the 123 patients who achieved a second complete remission (CR2) following salvage chemotherapy, 62 underwent allogeneic haematopoietic stem cell transplantation (Allo-HSCT) in CR2. The median duration between the achievement of CR2 and Allo-HSCT in CR2 was 68 d. The overall survival (OS) rate from CR2 was significantly better in patients who underwent Allo-HSCT in CR2, according to a landmark analysis that was limited to patients who were alive without disease at 68 d from the achievement of CR2.

Outcome of Allo-HSCT in non-remission after relapse

In relapsed patients who had received chemotherapy alone in CR1, 18 patients directly underwent Allo-HSCT after relapse without salvage chemotherapy and 46 underwent Allo-HSCT in non-remission (NR) following failed salvage chemotherapy. OS from Allo-HSCT in patients who underwent Allo-HSCT in NR after salvage chemotherapy was 39·1% at 1 year and 20·7% at 5 years. OS from Allo-HSCT in patients who underwent Allo-HSCT directly after relapse was 38·9% at 1 year and

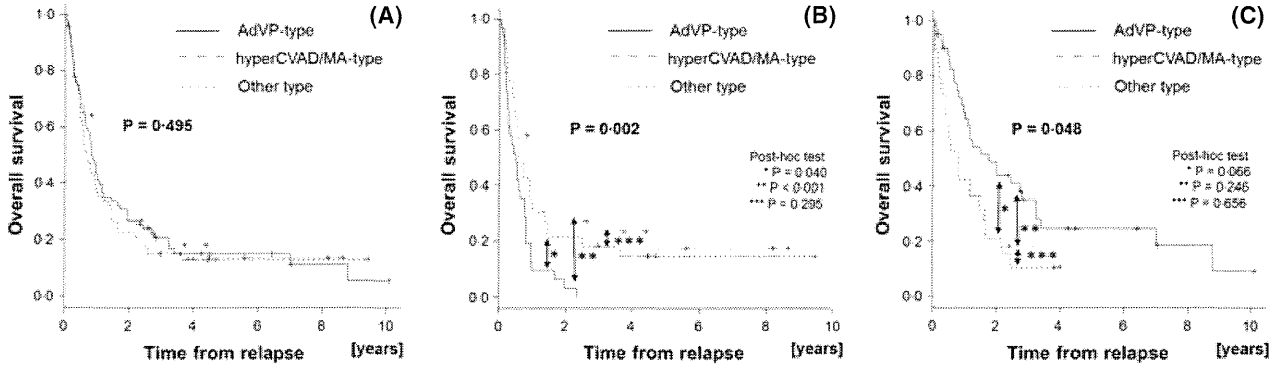

Fig 4. Overall survival from relapse in patients who received chemotherapy alone in the first complete remission according to the salvage chemotherapy regimen. The type of salvage regimen did not significantly influence overall survival (OS) from relapse (A). However, if patients were stratified into two groups according to the duration of the first complete remission (CR1), the type of salvage regimen was significantly associated with OS. In patients who had a shorter duration of CR1 (<1 year), an AdVP-type regimen (doxorubicin, vincristine, and predonisone-based) was associated with a worse OS (B), and in patients who had a longer duration of CR1 (more than 1 year), an AdVP-type salvage regimen tended to result in a better OS (C). hyperCVAD/MA, fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high doses of methotrexate and cytarabine.

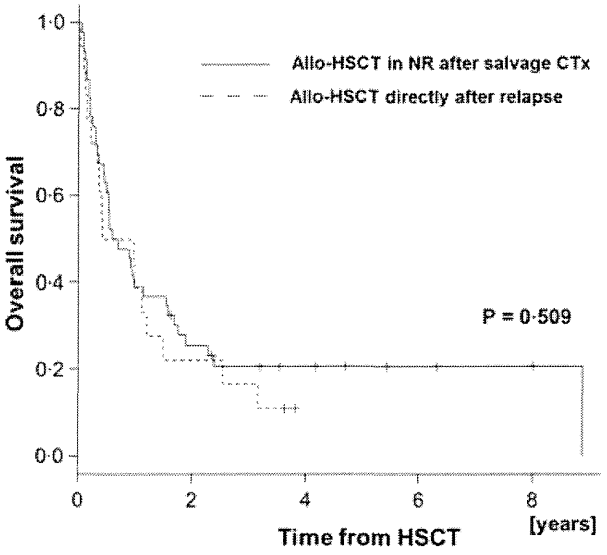

Fig 5. Overall survival from allogeneic haematopoietic stem cell transplantation in non-remission after relapse. In relapsed patients who received chemotherapy alone in the first complete remission, there was no difference in overall survival from allogeneic haematopoietic stem cell transplantation (Allo-HSCT) between patients who underwent Allo-HSCT in non-remission (NR) following salvage chemotherapy (CTx) and patients who underwent Allo-HSCT directly after relapse without salvage CTx.

11·1% at 5 years. There was no difference in OS from Allo-HSCT between these two groups (HR: 0·81, 95% CI: 0·17–1·45, $P = 0·51$; Fig 5).

Discussion

Several large clinical studies have demonstrated the outcomes of a relatively large number of patients with ALL who relapsed after uniform chemotherapy regimens (Fielding *et al*, 2007; Tavernier *et al*, 2007; Oriol *et al*, 2010; Gokbuget *et al*, 2012), and the results of uniform salvage chemotherapy regimens have been reported as clinical trials, each of which included a small number of relapsed patients with ALL (Giona *et al*, 1997; Koller *et al*, 1997; Montillo *et al*, 1997; Weiss *et al*, 2002; Camera *et al*, 2004; Specchia *et al*, 2005). The present study investigated the prognosis of relapsed patients with Ph-negative ALL, based on the clinical data of 332 patients from 69 institutions all over Japan. These patients had received various kinds of treatment strategies before and after relapse according to their respective institution, and therefore, these patients should reflect the more general population of relapsed patients with ALL.

Overall survival (OS) at 5 years from relapse was 16·3% in patients who received chemotherapy alone in CR1 and 10·6% in patients who received Allo-HSCT in CR1, and Allo-HSCT in CR1 did not influence the outcome after relapse in our study. These outcomes were comparable to those in three previous reports of large clinical studies (Fielding *et al*, 2007; Tavernier *et al*, 2007; Oriol *et al*, 2010; Table III). Recently, Gokbuget *et al* (2012) reported the outcome of 547 relapsed patients in the German Multicentre Study Group for Adult ALL (GMALL). The OS at 5 years from relapse in patients who had received chemotherapy alone in CR1 was significantly better than that in patients who had received Allo-HSCT in CR1 (28% vs. 15%, $P < 0·001$). This difference might be attributed to the high rate (75%) of Allo-HSCT after relapse in patients who had received chemotherapy alone in CR1. In our study, Allo-HSCT was performed in 55% of the patients who relapsed after they had received chemotherapy alone in CR1. Allo-HSCT in CR2 was associated with a better prognosis after the achievement of CR2, and in addition, some of the patients who received Allo-HSCT even in NR after salvage chemotherapy showed long-term survival (the OS was 20·7%