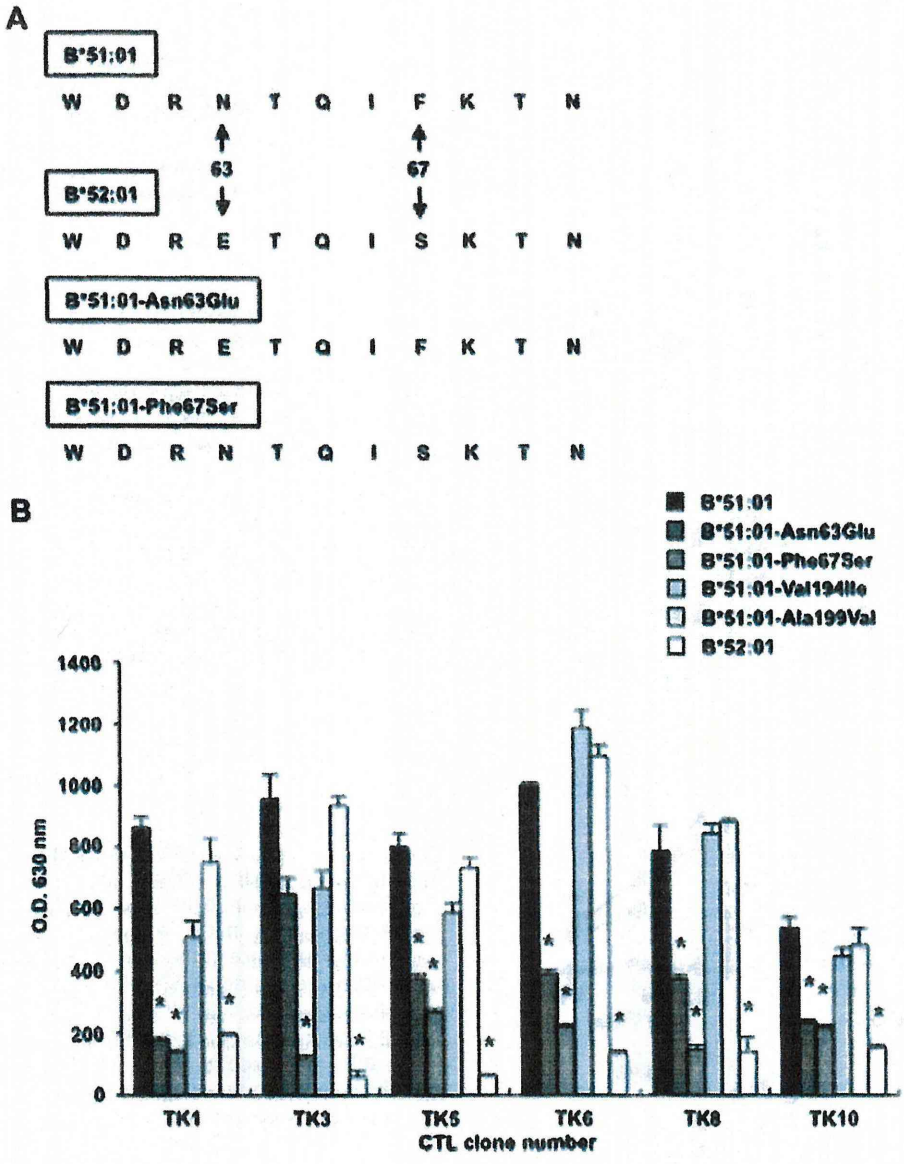

Fig. 4. Recognition of the HLA-B*51:01 molecule by CTLs is peptide-dependent. (A) The amino acid sequences at position 60 to 70 of the *B*51:01*, *B*52:01*, *B*51:01-Asn63Glu*, and *B*51:01-Phe67Ser* cDNAs are shown. Asn at position 63 was substituted with the corresponding amino acid in B*52:01, Glu, in the *B*51:01-Asn63Glu* mutant. Phe at position 67 was substituted with the corresponding amino acid in B*52:01, Ser, in the *B*51:01-Phe67Ser* mutant. (B) COS cells were transfected with a plasmid encoding *B*51:01*, *B*51:01-Asn63Glu*, *B*51:01-Phe67Ser*, *B*51:01-Val194Ile*, *B*51:01-Ala199Val* or *B*52:01* cDNA construct, cocultured with CTL clones, and IFN-γ production was measured in the supernatant using ELISA. Data are the means and SD of triplicate determinations. *Significant difference ($p < 0.05$: Student's *t*-test) in the IFN-γ production stimulated by each mutant or *B*52:01* cDNA construct compared with the wild-type *B*51:01* cDNA construct. Data are representative of three experiments.

(Fig. 3A), and *B*51:01*-transfected donor B-LCL, which are derived from B lymphocytes, were lysed by CTLs (Fig. 3B).

3.5. Leukemia blasts escaped from immunological pressure by HLA-B-specific CTLs

Whether the leukemia blasts escaped from the cytotoxicity of HLA-B*51:01-specific CTL clones was then examined. Pre-transplant and post-transplant leukemia blasts were purified by fluorescence-activated cell sorter (purity, ~62% and ~99%, respectively), and a cytotoxicity assay was performed only for the TK1 CTL clone because of the limited number of cryopreserved blasts. Weak but clear lysis of pre-transplant leukemia blasts by the TK1 CTL clone was observed, whereas post-transplant leukemia blasts were not lysed (Fig. 5A). All other CTL clones (TK3, TK5, TK6, TK8, and TK10) also did not lyse post-transplant leukemia blasts (Fig. 5B).

In addition, whether HLA-B*51:01-specific CTL pressure persisted until leukemia relapse was examined. The IFN-γ ELISPOT assay was performed to detect HLA-B*51:01-reactive CTLs in patient blood on day 232, 1 month before clinical leukemia relapse (Fig. 6). IFN-γ-producing B*51:01-reactive T lymphocytes were detected at a level nearly equal to the level of recipient B-LCL-reactive CTLs, that is, the total CTL alloresponse.

4. Discussion

The mechanism of leukemia relapse in this recipient can be explained as follows. CTLs specific for HLA-B*51:01 molecule/

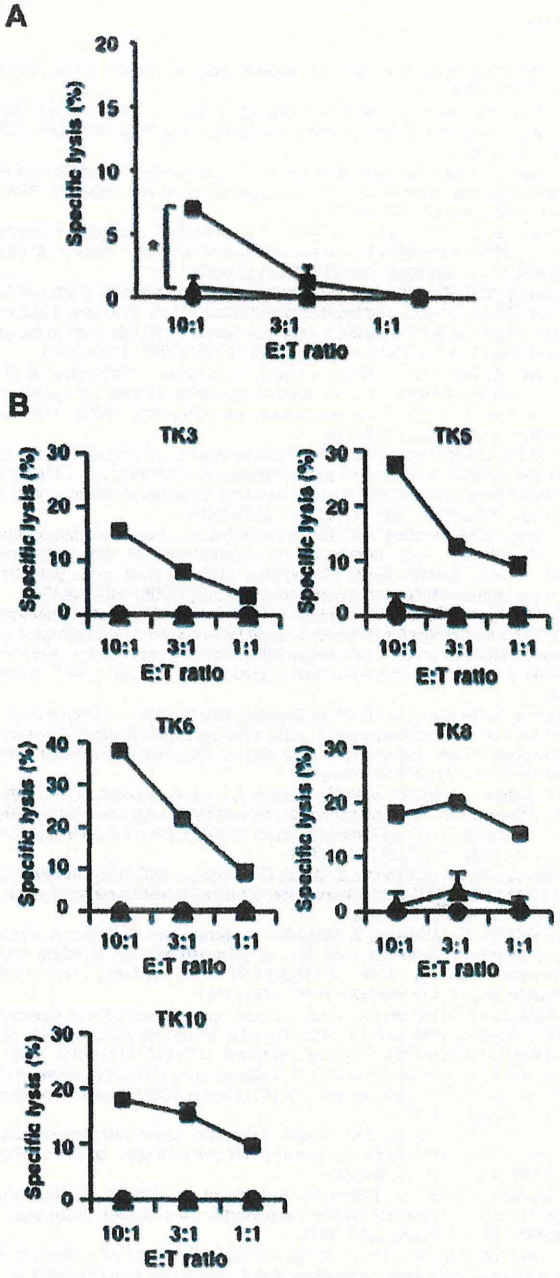

Fig. 5. Cytotoxicities of CTLs against leukemia blasts. (A) Purified pre-transplant leukemia blasts (purity, ~62%) (square), purified post-transplant leukemia blasts (purity, ~99%) (triangle) and donor B-LCL (circle) were used as targets for TK1 CTL clones. Specific lysis is shown as the mean and SD of triplicate cultures at various E:T ratios. *Significant difference ($p = 0.024$: Student's t-test) in the lysis of the pre-transplant leukemia blasts compared with the post-transplant leukemia blasts. Data are representative of three experiments. (B) Purified post-transplant leukemia blasts (purity, ~99%) (triangle), B-LCLs from the patient (square) and donor (circle) were used as targets for CTL clones. Specific lysis is shown as the mean and SD of triplicate cultures at various E:T ratios. Data are representative of three experiments. There was no significant difference in the lysis of the post-transplant leukemia blasts compared with B51-negative donor B-LCL (negative control).

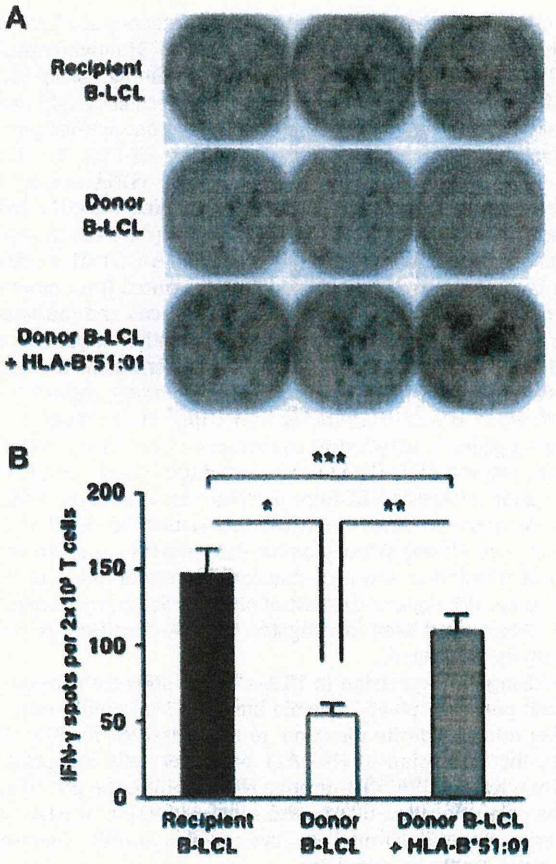

Fig. 6. Detection of HLA-B*51:01-specific CTLs in T lymphocytes obtained from the recipient on day 232 after transplantation. (A) Representative ELISPOT wells show triplicate results of T lymphocytes stimulated by recipient B-LCL, donor B-LCL, and *HLA-B*51:01*-transfected donor B-LCL. Data are representative of three experiments. (B) The frequency of CTLs in T lymphocytes recognizing the HLA-B*51:01 molecule was measured by IFN-γ ELISPOT analysis. The frequency of IFN-γ-producing cells is shown against recipient B-LCL (black), donor B-LCL (white), and *HLA-B*51:01*-transfected donor B-LCL (gray). Data are the means and SD of triplicate determinations. *$p = 0.0057$; **$p = 0.0077$; ***$p = 0.090$ (Student's t-test). Data are representative of three experiments.

non-leukemia peptide complex were generated in the recipient blood during acute GVHD, and these CTLs continued to produce immunological pressure on leukemia blasts for at least 8 months after transplantation, but B*51:01-down-regulated leukemia blasts escaped from the pressure of B*51:01-specific CTLs, and then the leukemia clinically relapsed.

CTLs recognizing mismatched HLA molecules play an important role in the immune reaction after HLA-mismatched HSCT, including graft rejection [25–27], GVHD [28], and the GVL effect [11]. In this study, the mismatched HLA-B*51:01-specific CTLs could participate both in GVHD and the GVL effect in the recipient. Ten CTL clones were isolated from the recipient's blood just after the onset of grade III acute GVHD involving skin, gut, and liver, and all clones demonstrated HLA-B*51:01-specific cytotoxicity in a non-leukemia peptide-dependent manner (Fig. 3 and 4). The patient was suffering from GVHD until his death on day 279, and in the ELISPOT assay for T lymphocytes obtained from recipient blood on day 232, HLA-B*51:01-reactive T lymphocytes accounted for the majority of alloreactive T lymphocytes (Fig. 6). Meanwhile, weak but clear lysis of pre-transplant leukemia blasts by an HLA-B*51:01-specific CTL clone was confirmed (Fig. 5A), and the primary refractory T lymphoblastic leukemia/lymphoma was in remission until day 261. These data are consistent with participation of the recipient HLA-B locus-specific CTLs both in GVHD and the GVL effect.

Selective HLA down-regulation was seen in this patient's post-transplant leukemia blasts. Mechanisms that alter HLA class I expression have been investigated and summarized as follows [29]: (1) loss of heterozygosity in chromosome 6 and/or 15, in

which class I heavy chain or β_2-microglobulin genes are located [30,31]; (2) mutations in these genes [32]; and (3) down-regulation of the antigen processing machinery, including transporter associated with antigen processing and low-molecular-weight protein genes [33]. Ten *HLA-B* cDNAs were cloned from purified post-transplant leukemia blasts (purity, ~99%) by RT-PCR; 5 (50%) clones were identical to the canonical *B*51:01* cDNA sequence, and the others (50%) were identical to the canonical *B*54:01* cDNA sequence, which was another recipient B allele (data not shown). These data suggest that down-regulation of HLA-B*51:01 expression in the post-transplant leukemia blasts resulted from mechanisms other than loss of heterozygosity of B locus and mutation of the *B*51:01* gene itself, although the entire sequence of *B*51:01* DNA including introns has not been determined. Recently, hypermethylation of the HLA-class I gene promoter regions has been identified as a mechanism for transcriptional inactivation of HLA class I genes in esophageal squamous cell carcinoma lesions [34]. We analyzed *B*51:01* promoter methylation by pyrosequencing of bisulfite-treated DNA from purified post-transplant leukemia blasts and confirmed no hypermethylation of the *B*51:01* gene (data not shown). Other possible mechanisms are down-regulation of translation and post-translational modification of the *B*51:01* gene, although, to the best of our knowledge, these mechanisms have not yet been investigated for HLA-class I genes. Further analysis is required.

The change in expression in HLA-A*11:01 between pre-transplant and post-transplant leukemia blasts was of similar magnitude, but in the opposite direction, to that observed for B*51:01. Because the expression of HLA-A11 on target cells can protect them from lysis by KIR3DL2-positive NK cells [35], the possibility that the post-transplant blasts with high expression of HLA-A11 were resistant to NK cell-mediated cytotoxicity, resulting in leukemia relapse, cannot be ruled out.

A question left unresolved is whether the present observation is unique to this recipient or can be duplicated in additional recipients who receive HLA one locus-mismatched HSCT. However, the present findings can explain, at least in part, the mechanism of how leukemia relapse occurs during persistent GVHD after HSCT. Another question is whether the present observation is unique to T lymphoblastic leukemia/lymphoma, which is a relatively rare subset of acute leukemia in adults. The relevance of this finding to other leukemias, including B lymphoblastic leukemia/lymphoma and myeloid malignancies, should be confirmed. Further efforts to identify the peptides that are presented by HLA-B*51:01 molecules and recognized by isolated CTL clones should help to elucidate the precise mechanisms of leukemia escape.

In conclusion, immune escape of leukemia blasts from CTL pressure toward a mismatched HLA molecule/non-leukemia peptide complex may lead to clinical leukemia relapse.

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tions, and the germline sets (A and C) have the advantage of not requiring DNA sequencing or specific custom-made primers.

Two DNA MRD markers with high sensitivity (at least 10^{-4}) are generally required in MRD intervention clinical trials,[1,9] and in a large cohort of 2854 pediatric precursor B ALL patients, 20% of patients had only one sensitive marker and 8% had none.[9] Four of the 16 cases evaluated in this study had only one sensitive Ig/TCR marker so that availability of *IKZF1*-based MRD testing would have been useful for their risk stratification. Using routine PCR, *IKZF1*Δ3–6 rearrangements were identified in 6% of ALL patients in the ANZCHOG cohort in this study, so inclusion of this marker in standard screening for MRD targets would be an easy way to provide more patients with two sensitive markers.

The concept of using disease-related markers for MRD testing has been already established for fusion transcripts such as BCR-ABL and for gene rearrangements such as for *SIL-TAL1* in T-ALL and for *MLL* rearrangements in infant ALLs.[10] Kuiper *et al.*[4] in an analysis of paired diagnosis and relapse samples from 34 patients found *IKZF1* deletions and nonsense mutations in 14 (41%) patients at diagnosis and showed that all were conserved at relapse, in contrast to other recurrent genetic lesions found at diagnosis such as *PAX5*, *CDKN2A* and *EBF1*. It is therefore likely that this *IKZF1* marker will be at least as stable as Ig/TCR rearrangements, although this will need to be confirmed in more extensive studies.

In summary, we have assessed three ways to measure MRD levels by RQ-PCR for the most common deletion of the *IKZF1* gene found in ALL and demonstrated that all three methods provided robust and sensitive MRD assays for patients with this arrangement. The two primer and probe sets based on germline sequences could be used within a few days of diagnosis to provide quantitative measures of very-early responses to therapy. We expect that *IKZF1* gene deletions (*IKZF1*Δ3–6 and probably others) will provide a useful addition to the repertoire of MRD markers currently available for monitoring MRD in ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

Prognostic factors for acute myeloid leukemia patients with t(6;9)(p23;q34) who underwent an allogeneic hematopoietic stem cell transplant

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often selected as a curative treatment strategy for acute myeloid leukemia (AML). In particular, AML patients with poor cytogenetics at diagnosis are considered for allo-HSCT as the first-line therapy.[1–3] Recently, we have reported that AML with the t(6;9)(p23;q34) abnormality, which predicts a very poor prognosis in patients treated with chemotherapy,[4] is associated with an

outcome in patients receiving allo-HSCT that is comparable to that in patients with a normal karyotype.[5] However, 55% of the AML patients with t(6;9)(p23;q34) eventually had a negative outcome. We herein performed a further analysis for AML patients with t(6;9)(p23;q34) who received allo-HSCT to identify the prognostic factors affecting their overall survival (OS).

A total of 64 *de novo* AML patients with t(6;9)(p23;q34) detected in G-band staining at diagnosis, who received their first allo-HSCT between January 1996 and December 2007, were extracted from the databases of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Cord Blood Bank Network. The cytogenetic data were analyzed according to the South-western Oncology Group criteria for each institution, instead of by central review.[2] The clinical data were collected using a standardized report form, which was submitted at 100 days, 1 year and annually after HSCT. This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Written informed consent was obtained in accordance with the Declaration of Helsinki. The OS was defined as the number of days from HSCT until death from any cause. Non-relapse mortality (NRM) was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. The analysis was performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; www.r-project.org).[6] The probability of OS was calculated using the Kaplan–Meier method and compared using the log-rank test. The probabilities of transplant related mortality and disease relapse were compared using the Grey test[7] and were analyzed using the cumulative incidence analysis,[6] while considering relapse and death without disease relapse as respective competing risks. The following variables related to the survival of the adult patients older than 15 years and their clinical data were compared in a univariate analysis: recipient characteristics (age; younger than 35 vs. older than 35 years, gender, performance status at diagnosis; 0 to 2 vs. 3 or 4, FAB classification; M2 or others, positivity for peroxidase in leukemic blasts at diagnosis; less than 50% vs. greater than 50%, cytogenetic abnormality), donor characteristics (age; younger than 35 vs. older than 35 years, gender, sex compatibility, compatibility of cytomegalovirus antibody serostatus, relationship; related vs. unrelated, and ABO compatibility), transplant characteristics (disease status at HSCT; complete remission (CR) vs. non-CR, use of total body irradiation as a preconditioning regimen, source of the graft; bone marrow, peripheral blood stem cell, cord blood (CB)), graft-versus-host disease prophylaxis; cyclosporine versus tacrolimus and the use of methotrexate. Multivariate Cox models were used to evaluate the hazard ratios associated with the prognosis. Covariates found to be significant in the univariate analyses ($P \leqslant 0.10$) were included in the models. For both the univariate and the multivariate analyses, *P*-values were two sided, and outcomes were considered to be significant for $P \leqslant 0.05$.

The characteristics of the 64 AML patients with t(6;9)(p23;q34) were shown in Table 1a. The OS of the seven pediatric patients younger than 14 years old seemed to be better than the OS of the 57 adult patients older than 15 years, although there were no statistically significant differences between the groups (Figure 1a, the probability of 3-year OS in pediatric patients and adult patients was 83% and 48%, respectively ($P = 0.12$)). We performed a further analysis in the 57 adult patients older than 15 years. The univariate analysis showed that the disease status at HSCT was the sole significant prognostic factor affecting the OS (Figure 1b, the probability of 3-year OS in patients with CR and with non-CR at HSCT was 69% and 29%, respectively ($P < 0.003$)), and the number of HLA disparities, M2 in the FAB classification and CB as the source of the graft were calculated to have a *P*-value < 0.1 (Table 1b). No statistically significant tendencies related to gender, gender mismatch between the donor and recipient, recipient cytomegalovirus serostatus or the use of total body irradiation for the preconditioning regimen were observed. The cumulative incidence of relapse and of NRM are shown in Figure 1c; the cumulative incidence of relapse was significantly lower in patients with a CR at HSCT than in patients without CR, although such differences were not seen in the cumulative incidence of NRM between these two groups (the 3-year cumulative incidence of relapse was 25% in CR patients and 58% in non-CR patients ($P = 0.005$), and the 3-year cumulative incidence of NRM was 10% in CR patients and 16% in non-CR patients ($P = 0.85$)). In the multivariate analysis, the disease status at HSCT and FAB-M2 remained the significant variables associated with the OS (Table 1b). The OS of the patients categorized by the combination of the disease status at HSCT and FAB-M2 showed a favorable outcome in FAB-M2 patients with a CR at HSCT (Figure 1d, the probability of 3-year OS in patients with CR/FAB-M2, CR/non-FAB-M2, non-CR/FAB-M2 and non-CR/non-FAB-M2 was 76%, 60%, 43% and not reached, respectively ($P < 0.001$)). In contrast, the patients who

Table 1a. Characteristics of patients with t(6;9)(p23;q34)

	Children (n = 7)	*Adult (n = 57)*
Age, median (range)	9 (6–14)	35 (17–58)
Gender, male/female	1/6	34/23
FAB classification[a]		
M0	0	1
M1	0	7
M2	5	32
M4	1	13
M5	1	2
Status at HSCT, CR/non-CR	5/2	29/28
HLA disparity[b]		
0	2	24
1	2	5
2	0	10
Graft source		
BM	3	32
PBSC	2	12
CB	2	13

Abbreviations: BM, bone marrow; CB, cord blood; CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; PBSC, peripheral blood stem cells. [a]Data not available in 2 adult patients. [b]Data not available in 3 pediatric patients and 18 adult patients.

Table 1b. Prognostic factors affecting overall survival of adult patients with t(6;9)(p23;q34)

Variables	*Risk factors*	*Univariate*	*Multivariate*		
			HR	*95% CI*	*P-value*
Disease status at HSCT	CR Non-CR	<0.003	1 2.54	1.17-5.51	<0.02
FAB classification	M2 other than M2	0.075	1 3.61	1.59–8.21	<0.003
Number of HLA disparity	0 1 2	0.061		NA	
Source of the graft	BM or PBSC CB	0.076		NA	

Abbreviations: BM, bone marrow; CB, cord blood; CI, confidence interval; CR, complete remission; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; NA, not assessed; PBSC, peripheral blood stem cell.

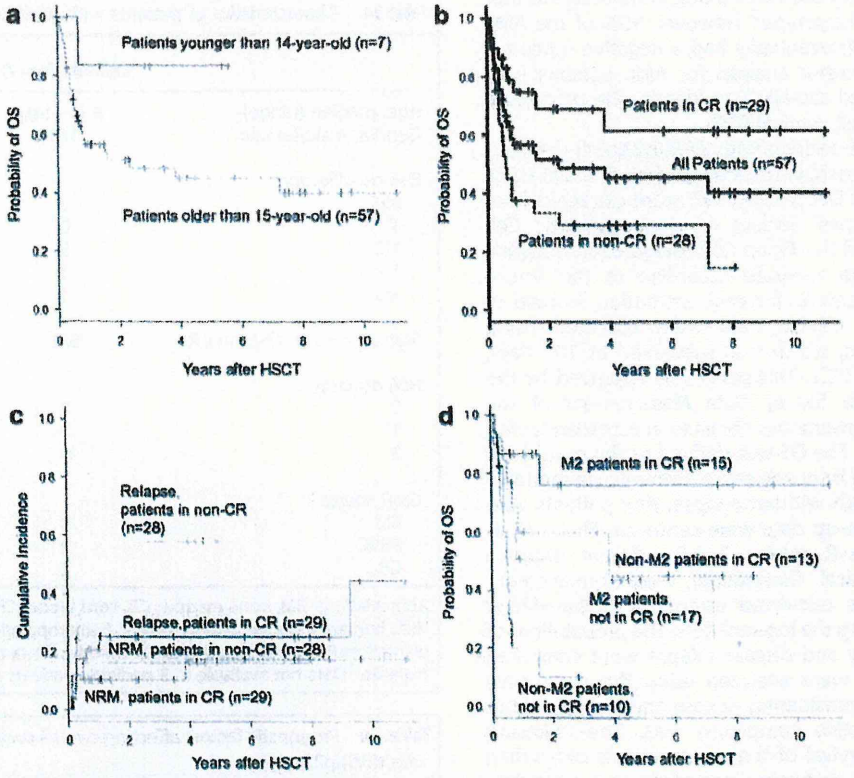

Figure 1. (**a**) The probabilities of OS in the patients with the t(6;9)(p23;q34) abnormality, stratified by age at HSCT. Solid line, pediatric patients younger than 14 years; dotted line, adult patients older than 15 years. (**b**) The probabilities of OS of the patients older than 15 years, stratified by the disease status at HSCT. Bold line, all patients; solid line, patients in CR; dotted line, patients in non-CR. (**c**) The cumulative incidence of events after allo-HSCT stratified by the disease status at the time of HSCT. Solid line, cumulative incidence of relapse of the patients in CR; dashed line, cumulative incidence of NRM of the patients in CR; dotted line, cumulative incidence of relapse of the patients in non-CR; chain line, cumulative incidence of NRM of the patients in non-CR. (**d**) The probability of OS of the patients grouped according to the FAB classification and the disease status at HSCT. Solid line, FAB-M2 patients in CR; dashed line, non-FAB-M2 patients in CR; dotted line, FAB-M2 patients in non-CR; chain line, non-FAB-M2 in non-CR.

were not in remission at the time of HSCT and had non-FAB-M2 showed a poorer outcome; the cause of death in six out of the nine patients was due to a relapse of the AML.

The characteristics of the patients with the t(6;9)(p23;q34) subtype of AML were known to have a poor prognosis and to be associated with development at a younger age, frequent M2 in the FAB classification and achievement of a morphological first CR not predicting a favorable outcome.[8] In this study, we distinguished the seven pediatric patients who seemed to have a superior OS from the adult patients, because the better prognosis in the children might reflect differences in the pathogenesis of the disease, consistent with the better OS in the previous report.[4] The current study revealed that the cumulative incidence of relapse was significantly worse in patients without CR than in patients with CR, although the cumulative incidence of NRM was comparable between these two groups. These results indicate that it is important to have an appropriate treatment strategy, that is, allo-HSCT for the patients who achieved first CR is imperative, while the development of an effective treatment for the refractory/relapsed AML patients is critical. The presence of FLT3-ITD is recognized as a poor prognostic factor in AML patients.[9] As FLT3-ITD was frequently detected in patients with t(6;9)(p23;q34),[4] it has been suggested that the presence of FLT3-ITD might contribute to the poor prognosis of the t(6;9)(p23;q34) patients.[10] With regard to the rate of FLT3-ITD−positive disease, there was no apparent between-group differences in the FAB classification;[11] however, the expression levels of FLT3 were higher in patients with monocytic AML (M4 and M5 in the FAB classification) than in the other patients,[12] and were associated with an unfavorable prognosis.[13] The current study has distinguished FAB-M2 from non-M2, and two-thirds of the non-M2 cases ($n=23$) in this study consisted of monocytic AML (the number of M4 patients and M5 patients was 13 and 2, respectively). Therefore, the poor prognosis of the non-FAB-M2 patients might be due to the presence of FLT3-ITD. Unfortunately, we could not confirm this hypothesis because this retrospective analysis did not examine the presence of FLT3-ITD. Future studies will be needed to determine whether the FLT3-ITD status was responsible for the poor prognosis in these patients.

In conclusion, this study showed that a CR at the time of HSCT and M2 in the FAB classification are favorable prognostic factors in AML patients with t(6;9)(p23;q34). However, refractoriness to chemotherapy remains an obstacle to a favorable allo-HSCT outcome, especially in non-M2 patients. Novel therapeutic approaches, such as immunotherapy using anti-FLT antibodies combined with HSCT, may also be required for patients expected to have a poor prognosis.[14,15]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Polymorphisms in xenobiotic transporters *ABCB1*, *ABCG2*, *ABCC2*, *ABCC1*, *ABCC3* and multiple myeloma risk: a case–control study in the context of the International Multiple Myeloma rESEarch (IMMEnSE) consortium

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Multiple myeloma (MM) is a hematological neoplasm that arises from a single clone of malignant plasma cells in the bone marrow. In Europe, 4.6/100 000 males and 3.2/100 000 females every year develop MM, with a median age at diagnosis around 60 years.[1]

The observation of a higher risk to develop MM among first-degree relatives of MM patients in several population-based case–control studies supports the idea that genetic factors are involved in MM pathogenesis.[2] Therefore, several studies focusing on various genes and pathways have tried to identify single-nucleotide polymorphisms (SNPs) associated with the susceptibility to the disease.[3,4]

The detoxification and elimination of xenobiotic compounds is one of the most investigated processes in cancer susceptibility, with several evidences of its association with cancer risk.[5] ATP-binding cassette (ABC) subfamily B, member 1 (*ABCB1* or *MDR1*); subfamily G, member 2 (*ABCG2* or *BCRP*); subfamily C, member 2 (*ABCC2* or

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Peripheral Blood as a Preferable Source of Stem Cells for Salvage Transplantation in Patients with Graft Failure after Cord Blood Transplantation: A Retrospective Analysis of the Registry Data of the Japanese Society for Hematopoietic Cell Transplantation

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To compare the different stem cell sources used in salvage transplantation for graft failure (GF) after cord blood transplantation (CBT), we retrospectively analyzed data of 220 patients who developed GF after undergoing CBT between January 2001 and December 2007 and underwent a second hematopoietic stem cell transplantation (HSCT) within 3 months. The donor sources for salvage HSCT were cord blood (n = 180), peripheral blood stem cells (PBSCs; n = 24), and bone marrow (BM; n = 16). The cumulative incidence of neutrophil engraftment on day 30 after the second HSCT was 39% with CB, 71% with PBSCs, and 75% with BM. Multivariate analysis revealed that PBSC and BM grafts were associated with a significantly higher engraftment rate than CB (hazard ratio [HR], 7.77; $P < .001$ and HR, 2.81; $P = .016$, respectively). Although the incidence of grade II-IV acute graft-versus-host disease was significantly higher in the PBSC group than in the CB group (HR, 2.83; $P = .011$), the incidence of 1-year nonrelapse mortality was lower in the PBSC group than in the CB group (HR, 0.43; $P = .019$), and 1-year overall survival was superior in the PBSC group compared with the CB group (HR, 0.45; $P = .036$). Our results suggest that PBSC is the preferable source of stem cells in salvage HSCT for GF after CBT.

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KEY WORDS: Engraftment, Second transplant, Peripheral blood stem cells

INTRODUCTION

Graft failure (GF) is one of the lethal complications of allogeneic hematopoietic stem cell transplantation (HSCT) [1]. Recently, the incidence of GF has increased with the introduction of cord blood transplantation (CBT), HLA-mismatched transplantation, and reduced-intensity conditioning (RIC)

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regimens. The GF rate after HSCT with conventional myeloablative conditioning (CST) regimens is <5% for peripheral blood stem cell transplantation (PBSCT), ~5%-10% for bone marrow transplantation (BMT), and ~20% for CBT [2-5]. Rocha et al. [4] reported GF rates of 10% for unrelated BMT and 22% for CBT. Atsuta et al. [5] reported GF rates of 6% and 23% in patients with acute myelogenous leukemia (AML) treated with unrelated BMT and CBT, respectively, and corresponding GF rates of 3% and 20% in patients with acute lymphoblastic leukemia (ALL). These results indicate that GF is not a rare complication.

The survival rate of patients who developed GF after allogeneic HSCT but did not undergo a second salvage transplantation has been <10% [4-6]. In a recent investigation of the feasibility of a second HSCT with an RIC regimen for GF, Guardiola et al. [7] reported the clinical outcomes of 82 patients with GF who underwent a second transplantation, most from an HLA-matched sibling donor. The 3-year overall survival (OS) rate in these patients was 30%, significantly better than that reported in a previous study in which the patients did not undergo a second HSCT. Waki et al. [8] analyzed the clinical outcomes of 80 patients with GF who underwent salvage CBT and found a 1-year OS rate of 33%, with nonrelapse mortality (NRM), especially infectious diseases, as the major cause of death [8]. This OS rate was higher than that reported in a previous study in which patients did not undergo a second HSCT; however, the low engraftment rate and high NRM rate after salvage CBT are not acceptable results. Schriber et al. [9] obtained clinical data of 122 patients who underwent unrelated HSCT as salvage treatment for GF from the database of National Marrow Donor Program. The engraftment rate of 74% in this series was markedly greater than that reported by Waki et al. [8], possibly attributable to differences in stem cell sources. However, the high engraftment rate did not improve the outcome because of a high NRM rate (86%). Infectious disease was the major cause of death in that study.

Given that infectious diseases are a main cause of failure after second HSCT, peripheral blood stem cells (PBSCs) may be the preferred stem cell source for the second transplantation. However, acute and chronic graft-versus-host disease (GVHD) is a concern after PBSCT, especially after HLA-mismatched transplantation. The effect of the stem cell source in a second HSCT has not yet been clarified, because previous studies had insufficient statistical power to analyze the effect of this factor using multivariate analysis.

In the present study, we retrospectively analyzed the clinical outcomes of patients who had undergone salvage HSCT after GF, using registry data of the Japanese Society for Hematopoietic Cell Transplantation (JSHCT). We compared outcomes by stem cell source: PBSCs, cord blood (CB), or bone marrow (BM).

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board of Izumisano Municipal Hospital, Rinku General Medical Center, Osaka, Japan. The patients' clinical data were obtained from the JSHCT database [10]. The following patients were included in the study: (1) patients who developed primary or secondary GF after allogeneic HSCT performed between January 2000 and December 2007, (2) patients who underwent a second HSCT for GF within 3 months after the diagnosis of GF, and (3) patients who did not demonstrate progression of the primary disease before the second HSCT. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) >500/mm^3 in the first 3 consecutive days after HSCT. Primary GF was defined in accordance with a previous report as an ANC not exceeding 500/mm^3 or the absence of donor T cells (<5%) before relapse, disease progression, second HSCT, or death [11]. Secondary GF was defined as a decrease in ANC of <100/mm^3 at 3 determinations or absence of donor T cells (<5%) after the initial engraftment without recovery before relapse, disease progression, second HSCT, or death. Chimerism was assessed using polymerase chain reaction for short tandem repeats or variable number tandem repeats. Sex chromosome chimerism in sex-mismatched donor–recipient pairs was assessed using fluorescence in situ hybridization.

Data of 382 patients were obtained from the JSHCT database. Of these 382 patients, 67 were excluded because of relapse or disease progression before the second HSCT, autologous hematopoietic recovery, autologous HSCT, or missing data on, for example, stem cell source, engraftment, and OS. Furthermore, patients who received PBSCs (n = 24) or BM (n = 71) for the first HSCT were excluded, to focus on CBT in this study. The data of the remaining 220 patients were subjected to further analysis.

Statistical Analysis

We first performed a statistical analysis of the differences in the 3 stem cell sources. Patients and transplantation characteristics in the different groups were compared using the χ^2 test to determine the difference in proportions. One-way analysis of variance was used to compare mean values. The primary endpoint of this study was the engraftment rate after second HSCT. Secondary endpoints included the probabilities of OS and progression-free survival (PFS) and the cumulative incidents of NRM, acute GVHD, and infectious diseases after the second HSCT. OS and PFS were estimated using the Kaplan-Meier method. The cumulative incidents of engraftment, relapse, GVHD, and infectious diseases were evaluated using