TABLE I. Patient Characteristics

	HCV-positive	%	HCV-negative	%	*P*
Total number	136		7,695		
Age, years, median (range)	49 (18–73)		47 (16–82)		0.11
<50	69	51	4,269	55	
≥50	67	49	3,426	45	0.3
Gender					
Male	91	67	4,511	59	
Female	45	33	3,184	41	0.053
Disease AML	55	40	3,109	40	
ALL	12	9	1,284	17	
MDS	21	15	792	10	
MPN_CML	6	4	345	4	
Other leukemias	1	1	59	1	
Lymphomas	34	25	1,571	20	
MM_PCN	1	1	134	2	
AA_PNH_PRCA	6	4	285	4	
Other diseases	0	0	116	2	0.12
Disease risk					
Standard	81	60	4,667	61	
High	55	40	2,982	39	0.72
Missing	0	0	45	0.6	
Prior SCT					
No	126	93	7,174	93	
Autologous or syngeneic	10	7	521	7	0.73
Performance status					
0 to 1	111	82	6,450	84	
2 to 4	19	14	857	11	0.34
Missing	6	4	388	5	
EBMT risk score					
0–1	7	5	469	6	
2–4	75	55	4,272	56	
5–7	52	38	2,809	37	0.9
Missing	2	1	145	2	
Sex match					
Match	62	46	3,760	49	
Female to male	41	30	1,711	22	
Male to female	24	18	1,711	22	0.08
Missing	9	7	513	7	
ABO					
Match	71	52	3,934	51	
Bidirectional mismatch	12	9	739	10	
Major mismatch	20	15	1,420	18	
Minor mismatch	32	24	1,579	21	0.64
Missing	1	0.7	23	0.3	
Donor source					
Related BMT	15	11	1,189	15	
Related PBSCT	31	23	1,488	19	
Unrelated BMT	64	47	3,260	42	
Unrelated CBT	26	19	1,734	23	0.28
Missing	0	0	24	0.3	
Serological HLA					
Match	89	65	4,923	64	
Mismatch	46	34	2,727	35	0.79
Missing	1	0.7	45	0.6	
Conditioning					
CYTBI ± α	42	31	2,937	38	
BUCY ± α	11	8	668	9	
Other MAC	21	15	955	12	
Flu-based RIC	55	40	2,817	37	
Other RIC	7	5	314	4	0.38
Missing	0	0	4	0.1	
GVHD prophylaxis					
CsA based	47	35	3,355	44	
Tac based	86	63	4,209	55	
Other	3	2	125	2	0.076
Missing	0	0	6	0.1	

"Other diseases" include EB virus-associated disease in 43, solid tumor in 21, hemophagocytic syndrome in 12, primary immunodeficiency in 21, congenital metabolic disorders in 2, and other in 17. High-risk diseases were defined as acute leukemia in the third or more complete remission or in nonremission; CML in the third or more chronic phase, in the accelerated phase, or in blastic crisis; lymphoma and MM in stable or progressive disease status; all plasma cell leukemia; adult T-cell leukemia/lymphoma in nonremission; and all solid tumors. All other diseases were classified as standard risk.

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; PCN, plasma cell neoplasms; AA, aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; PRCA, pure red cell aplasia; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; MAC, myeloablative conditioning; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; Tac, tacrolimus.

other hand, there was no difference in the related donor groups (91% vs. 93% in the HCV-positive and -negative groups, respectively ($P = 0.35$). In a multivariate analysis, the HCV serostatus did not remain significant.

The cumulative probabilities of platelet recovery of 50 $\times$ 10^9/L or higher at 60 days after HCT in the HCV-positive group (57% [95% CI: 48–65]) was significantly lower than that in the HCV-negative group (65% [95% CI: 64–66], $P = 0.012$, Fig. 2B). In subgroup analyses, the probability of platelet recovery at 60 days after HCT in the HCV-group was significantly lower among the unrelated BMT group (58% vs. 69%, $P = 0.029$), while there was no difference in the related donor group (71% vs. 75%, $P = 0.21$) and the unrelated CBT group (28% vs. 40%, $P = 0.13$). A multivariate analysis of the whole cohort revealed that HCV seropositivity was significantly associated with a lower rate of platelet recovery of 50 $\times$ 10^9/L or higher at 60 days after HCT (HR: 0.73 [95% CI: 0.59–0.92], $P = 0.0067$, Table II and Supporting Information Table I). In multivariate analyses of subgroups according to the donor source, the HCV seropositivity showed an increased risk for lower rate of platelet recovery in the related donors group (HR: 0.73, $P = 0.042$) and unrelated BMT group (HR: 0.72, $P = 0.047$). In unrelated CBT, the difference was not statistically significant (Table II).

Incidences of aGVHD, cGVHD, and SOS

Among the total 7,831 recipients, 2,821 recipients experienced Grades 2–4 aGVHD. The cumulative incidence of Grades 2–4 aGVHD was not different between the two groups (32% [95% CI: 24–40] in the HCV-positive group vs. 36% [95% CI: 35–37] in the HCV-negative group, $P = 0.19$). Among the 4,317 recipients who experienced aGVHD of any grade, the target organs were assessed in 4,305 for whom data were available. The HCV-positive group was significantly more likely to have liver aGVHD (24% vs. 14%, $P = 0.031$).

The 2,208 recipients who experienced cGVHD during the follow-up period were also analyzed. The cumulative incidences of cGVHD were not different between the two groups (31% [95% CI: 23–39] vs. 29% [95% CI: 28–30] at 2 years, $P = 0.66$). The target organs were assessable in 2,183 of the 2,208 recipients with cGVHD. With regard to liver cGVHD, there was no difference between the two groups (45% vs. 37%, $P = 0.33$).

The proportion of patients with SOS in the HCV-positive group (9 of 135 recipients, 7%) tended to be higher than that in the HCV-negative group (274 of 7,655 recipients, 4%, $P = 0.063$). Especially, when we focused on the recipients with MAC, SOS occurred significantly more frequently in the HCV-positive group (7 of 72 recipients, 10%) than in the HCV-negative group (179 of 4,495 recipients, 4%, $P = 0.026$). On the other hand, there was no difference in the incidence of SOS between the two groups among recipients with RIC (3% in each, $P = 0.71$). In a multivariate logistic analysis of the whole cohort, HCV did not remain a significant risk factor for the development of SOS.

Nonrelapse mortality

The cumulative incidence of NRM in the HCV-positive group (38% [95% CI: 28–48] at 2 years) was significantly higher than that in the HCV-negative group (25% [95% CI: 24–27] at 2 years, $P = 0.0063$, Fig. 3A). Notably, for patients aged 50 years or older, NRM in the HCV-positive group was significantly higher than that in the HCV-negative group (54% [95% CI: 36–68] vs. 32% [95% CI: 30–34] at 2 years, $P = 0.0039$). In contrast, differences in NRM according to HCV serostatus were not observed among younger recipients (28% vs. 20% at 2 years, $P = 0.18$).

In the analysis of subgroups stratified according to donor source, HCV seropositivity had no impact on NRM in the related HCT group (21% at 2 years, in each subgroup,

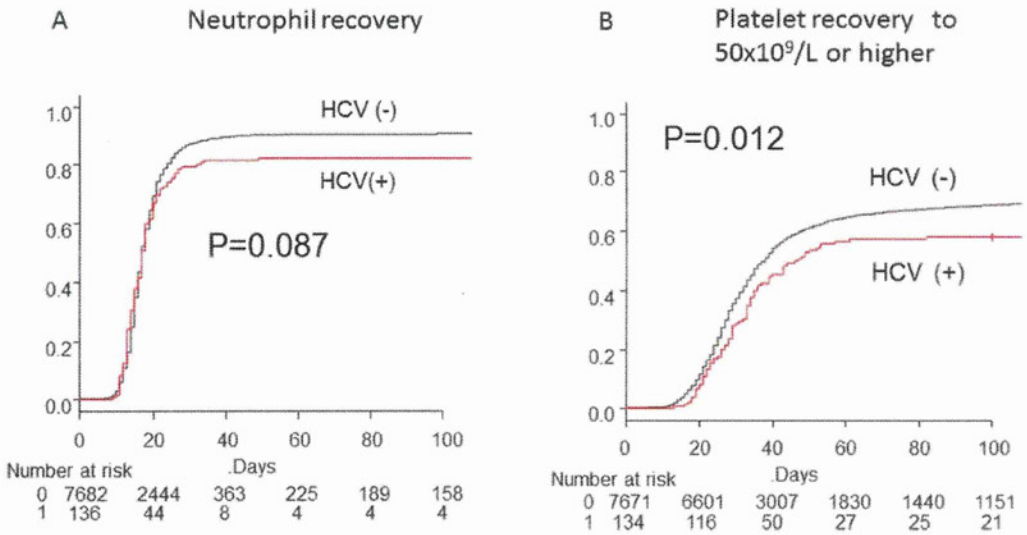

Figure 2. Comparison of the probabilities of hematopoietic recovery between the HCV-positive and -negative groups: (A) neutrophil engraftment and (B) platelet recovery to 50 × 10^9/L or higher. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$P = 0.88$, Fig. 3B). On the other hand, the adverse impact of HCV seropositivity on NRM was prominent in HCT from unrelated donors. In the unrelated BMT group, NRM in the HCV-positive group (46% [95% CI: 31–60] at 2 years) was significantly higher than that in the HCV-negative group (27% [95% CI: 25–29] at 2 years, $P = 0.0062$, Fig. 3C). Similarly, in the unrelated CBT group, NRM in the HCV-positive group (51% [95% CI: 23–73] at 2 years) tended to be higher than that in the HCV-negative group with borderline significance (31% [95% CI: 28–33] at 2 years, $P = 0.055$, Fig. 3D).

A multivariate analysis of the whole cohort revealed that HCV seropositivity was independently associated with a significantly increased risk of NRM after adjusting for age, gender, disease risk, performance status, EBMT score, the presence of prior autologous HCT, sex match, ABO match, HLA match, donor sources, and GVHD prophylaxis (HR 1.65 [95% CI: 1.19–2.30], $P = 0.0029$, Table II and Supporting Information Table I). In multivariate analyses of subgroups stratified according to donor source, the adverse impact of HCV seropositivity on NRM was observed only in HCT from unrelated donors (HR: 1.85 [95% CI: 1.23–2.80, $P = 0.0034$] in the unrelated BMT group and HR: 2.51 [95% CI: 1.19–5.31, $P = 0.016$] in the unrelated CBT group, Table II).

Overall survival

During the observation period, 3,648 of the 7,831 recipients died. The OS in the HCV-positive group (41% [95% CI: 32–50] at 2 years) was significantly lower than that in the HCV-negative group (51% [95% CI: 50–53] at 2 years, $P = 0.0070$, Fig. 4A). For patients aged 50 years or older, the OS in the HCV-positive group was significantly inferior to that in the HCV-negative group (22% [95% CI: 12–33] vs. 43% [95% CI: 41–45] at 2 years, $P < 0.0001$). No difference in OS was found among the younger recipients (60% vs. 58% at 2 years, $P = 0.55$).

In the analysis of subgroups stratified according to the donor source, the impact of HCV on OS was not seen in the related donor group (52% [95% CI: 36–66] at 2 years in the HCV-positive group vs. 54% [95% CI: 52–56] at 2 years in the HCV-negative group, $P = 0.76$, Fig. 4B). On the other hand, the adverse impacts of HCV on OS were significantly prominent in HCT from unrelated donors. In the unrelated BMT group, the OS in the HCV-positive group (39% [95% CI: 26–51] at 2 years) was significantly inferior to that in the HCV-negative group (55% [95% CI: 53–57] at 2 years, $P = 0.0054$, Fig. 4C). Similarly, in the unrelated CBT group, the OS in the HCV-positive group (28% [95% CI: 12–46] at 2 years) was also significantly inferior to that in the HCV-negative group (41% [95% CI: 39–44] at 2 years, $P = 0.039$, Fig. 4D).

A multivariate analysis of the whole cohort revealed that HCV seropositivity was independently associated with an significantly increased risk of inferior survival after adjusting for age, gender, age, disease, disease risk, performance status, EBMT score, sex match, HLA match, donor sources, conditioning regimen, and GVHD prophylaxis (HR: 1.39 [95% CI: 1.08–1.77], $P = 0.0096$, Table II and Supporting Information Table I). In multivariate analyses of

TABLE II. Impact of HCV-seropositivity on platelet recovery to 50 × 10^9/L or higher, NRM, and OS in a multivariate analysis

	Overall		Related donors		Unrelated BMT		Unrelated CBT	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Platelet recovery to 50 × 10^9/L or higher	0.73 (0.59–0.92)	0.0067	0.73 (0.53–0.99)	0.042	0.72 (0.52–1.00)	0.047	1.03 (0.53–1.99)	0.93
Nonrelapse mortality	1.65 (1.19–2.30)	0.0029	1.11 (0.52–2.39)	0.79	1.85 (1.23–2.80)	0.0034	2.51 (1.19–5.31)	0.016
Overall survival	1.39 (1.08–1.77)	0.0096	1.27 (0.81–1.96)	0.29	1.56 (1.13–2.17)	0.0076	1.78 (1.06–2.98)	0.029

HR of HCV serostatus was shown after adjusting for the factors of P less than 0.1 in univariate analysis with stepwise deletions among gender, age, disease, disease risk, performance status, EBMT score, the presence of prior autologous HCT, ABO match, sex match, HLA match, conditioning regimen, GVHD prophylaxis, and donor sources.

BMT, bone marrow transplantation; CBT, cord blood transplantation; HR, hazard ratio; CI, confidence interval.

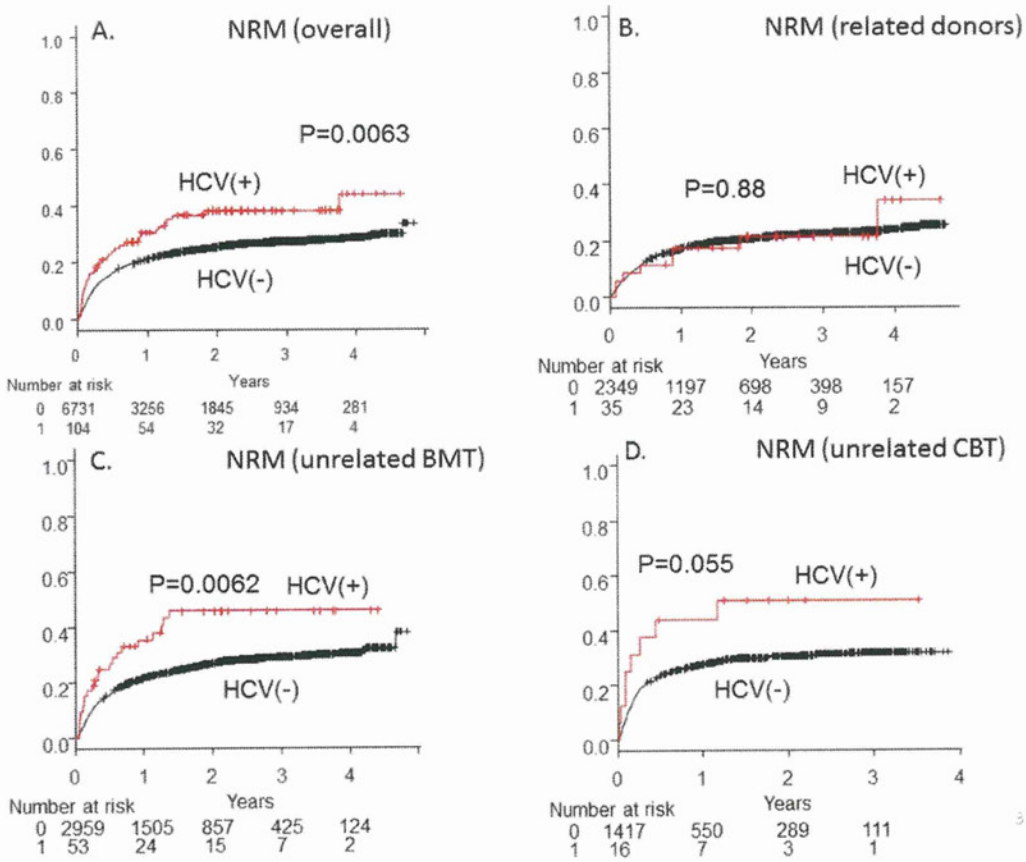

Figure 3. Comparison of the probabilities of NRM between the HCV-positive and -negative groups: (A) overall patients, (B) in the subgroup of related donors, (C) in the subgroup of unrelated BMT, and (D) in the subgroup of unrelated CBT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

subgroups stratified according to donor source, the adverse impact of HCV seropositivity remained significant, especially in HCT from unrelated donors (HR: 1.56 [95% CI: 1.13–2.17, $P = 0.0076$] in the unrelated BMT group and HR: 1.78 [95% CI: 1.06–2.98, $P = 0.029$] in the CBT group, Table II).

Causes of death

Overall, 77 (57%) of the 136 HCV-positive recipients and 3,571 (46%) of the 7,685 HCV-negative patients died during the follow-up period. The distributions of the causes of death seemed different between the two groups. The incidences of fatal hepatic problems in the HCV-positive group (8% vs. 2%, $P = 0.00034$) and fatal bacterial infection/sepsis (10% vs. 4%, $P = 0.0048$) were significantly higher than those in the HCV-negative group. In addition, the HCV-positive group had a trend of higher incidences of death due to graft-failure (5% vs. 2%, $P = 0.084$, Table III). Notably, among older recipients (50 years or older), the HCV-positive group had significantly higher incidences of fatal hepatic problems (12% vs. 3%, $P < 0.001$), fatal bacterial infection/sepsis (18% vs. 6%, $P < 0.001$), and death due to graft-failure (8% vs. 3%, $P = 0.046$). In contrast, there was no difference among younger recipients.

In an analysis of subgroups stratified according to the donor source, there was no significant difference in the incidences of fatal hepatic problems, death due to graft-failure, and fatal bacterial infection/sepsis in the related donor group (Table III). On the other hand, in the unrelated BMT group, the HCV-positive group showed a significantly higher incidence of fatal hepatic problems (14% vs. 3%, $P < 0.0001$, Table III). Furthermore, in the unrelated CBT group, HCV-positive patients had fatal bacterial infection (23% vs. 7%, $P = 0.0087$) and higher incidences of death due to graft-failure (15% vs. 4%, $P = 0.022$, Table III).

Regarding hepatocellular carcinoma, we did not found any death due to it during the short observational period.

Discussion

In this large cohort from a Japanese registry database, we showed that HCV seropositivity had adverse impacts on platelet recovery, NRM, and OS. Furthermore, this is the first to reveal that the impacts of HCV on NRM and OS differed according to the donor source and recipient age by subgroup analyses. HCV did not have an adverse impact on NRM or OS in HCT from related donors or in younger recipients, which was compatible with early studies [9–11]. On the other hand, HCV had prominent adverse effects on NRM and OS in unrelated BMT, unrelated CBT, and older recipients, which was compatible with a recent report from a Brazilian group that included unrelated donors and older recipients [14]. Therefore, we should pay attention to HCV seropositivity, especially in HCT from unrelated donors or for older recipients.

One of the reasons for the adverse impact of HCV on survival was the increased incidence of hepatic problems such as SOS and liver aGVHD. HCV is known to be a risk factor for severe SOS, although this has been controversial [9,11,23]. In our study, SOS was significantly more frequent in the HCV-positive group among recipients of MAC but not

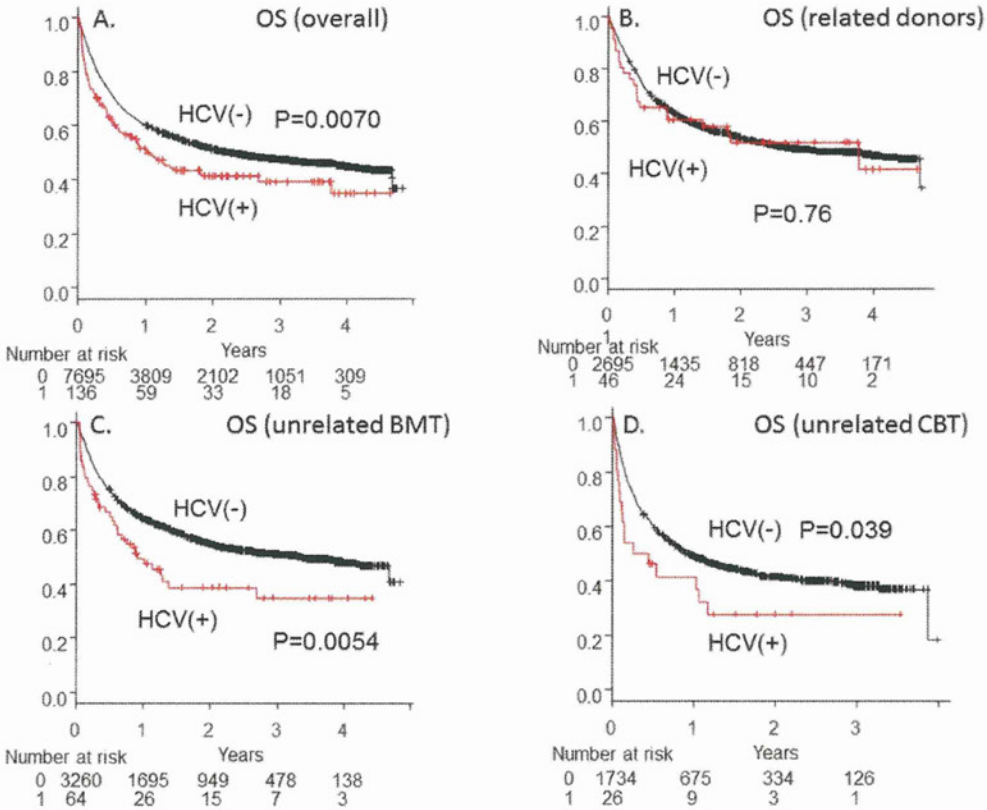

Figure 4. Comparison of the probabilities of overall survival between the HCV-positive and -negative groups: (A) overall patients, (B) in the subgroup of unrelated related donors, (C) in the subgroup of unrelated BMT, and (D) in the subgroup of unrelated CBT. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of RIC, which is at least partially because the recent use of RIC and the development of supportive therapies might reduce the risk for SOS [24]. In addition, HCV is also known to alter intrahepatic cytokine profiles and deteriorate hepatic injuries [25,26]. Therefore, hepatic problems, including SOS and liver GVHD, might tend to become severe and fatal in recipients with HCV. Another possible explanation is that prolonged immunosuppression, particularly in unrelated BMT, may enhance the replication of HCV and liver dysfunction, which may result in fatal hepatitis or liver failure [23,27,28]. Otherwise, reduced and dysfunctional T cells after HCT might not be able to suppress HCV reactivation. A long-term observation reported that transplant recipients with HCV developed liver cirrhosis more rapidly than nontransplant patients with HCV [13]. The adverse impact of HCV on hepatic problems including cirrhosis and hepatocellular carcinoma might become further prominent after long-term follow-up even in our cohort.

To the best of our knowledge, this study is the first to show that pretransplant HCV infection was associated with a lower rate of platelet recovery. Thrombocytopenia is frequently observed in HCV-infected patients with chronic hepatitis [29]. The presence of hypersplenism and a low thrombopoietin level are thought to be responsible for the thrombocytopenia in HCV-infected patients [30]. In addition, HCV is known to infect CD34-positive hematopoietic progenitor cells and to suppress their maturation like megakaryocytes [31,32]. These facts might also contribute to the delay of platelet recovery following HCT in the HCV-positive group.

The current analysis further suggested that recipients with HCV would be susceptible to fatal bacterial infections, especially unrelated CBT recipients. An excess of bacterial infections in HCT recipients with HCV has been reported previously [13,14], and a similar susceptibility to bacterial infection has also been described in HCV-positive patients who receive solid organ transplantation or dialysis [33,34]. HCV-infected patients have been reported to have dysfunctional phagocytes, T cells, and B cells [35,36], as well as the impaired maturation of hematopoietic progenitor cells [31,32]. These findings suggest that the defense mechanisms against bacterial infections are impaired in recipients with HCV. The appropriate strategy for preventing infection in recipients with HCV should be explored further.

This analysis had several limitations as a result of its retrospective nature, and all information was based on the reports by attending physicians, not on a central review. The HCV-positive group might include more patients with a worse disease status, such as those who had to receive HCT despite the presence of HCV infection and liver dysfunction, although there were no differences in the patient backgrounds including disease risk, performance status, and EBMT score before HCT. In addition, the registry database had no information on baseline liver functions, viral loads of HCV, pathological grades, and the presence of cirrhosis at clinical events, which are supposed to be important for risk stratification of HCV-positive patients. Therefore, the HCV-positive group might include more recipients with highly damaged liver functions such as cirrhosis. However, when we analyzed the association

TABLE III. Causes of death

	Overall			Related donors			Unrelated BMT			Unrelated CBT		
	HCV (136)	HCV-negative (7,695)	*P*	HCV (46)	HCV-negative (2,695)	*P*	HCV (64)	HCV-negative (3,260)	*P*	HCV (26)	HCV-negative (1,734)	*P*
Total death incidence	57% (77)	46% (3,571)	0.019	48% (22)	45% (1,222)	0.77	58% (37)	42% (1,383)	0.015	69% (18)	55% (962)	0.17
Fatal hepatic problems	8.1% (11)	2.2% (173)	0.00034	2.2% (1)	1.9% (52)	0.6	14% (9)	2.6% (85)	<0.0001	3.8% (1)	2.1% (36)	0.43
SOS	2.2% (3)	1.1% (82)	–	0.0% (0)	0.9% (24)	–	3.1% (2)	1.1% (37)	–	3.8% (1)	1.2% (21)	–
Liver aGVHD	0.7% (1)	0.1% (7)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (5)	–	0% (0)	0% (0)	–
Liver cGVHD	0% (0)	0.0% (2)	–	0.0% (0)	0.1% (2)	–	0% (0)	0% (0)	–	0% (0)	0% (0)	–
Hepatic failure due to uncertain cause	4.4% (6)	0.9% (71)	–	2.2% (1)	0.8% (22)	–	7.8% (5)	1.1% (36)	–	0% (0)	0.7% (13)	–
MOF with hepatic failure	0.7% (1)	0.1% (11)	–	0.0% (0)	0.1% (2)	–	1.6% (1)	0.2% (7)	–	0% (0)	0.1% (2)	–
Fatal bacterial infection	10% (14)	4.4% (336)	0.0048	6.5% (3)	3.2% (87)	0.19	7.8% (5)	3.9% (128)	0.11	23.1% (6)	7.0% (121)	0.0087
Death due to graft Failure	5.1% (7)	2.4% (187)	0.084	2.2% (1)	1.8% (48)	0.57	3.1% (2)	2.1% (68)	0.39	15% (4)	4.1% (71)	0.022

"–" indicates that statistical assessment was not performed in a subcategory of fatal hepatic problems.
BMT, bone marrow transplantation; CBT, cord blood transplantation; SOS, sinusoidal obstruction syndrome; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; MOF, multiple organ failure.

between HCV seropositivity and the presence of "hepatic-moderate/severe" in HCT-comorbidity index [37], on which we have started to gather information since 2008, we did not find any significant associations (data not shown), although HCV-seropositive patients in this cohort might be equal to patients with possible chronic HCV-hepatitis. On the other hand, strength of this study is that it involved the largest number of recipients with HCV of all studies to date and is the first to reveal the impact of HCV seropositivity on the clinical outcome of HCT in subgroups stratified according to the donor source. These findings in addition to liver functional tests could help a further risk stratification and management of HCV-positive recipients.

To date, the strategy against HCV infection, such as peginterferon and ribavirin therapy, has shown a sustained viral remission rate of close to 50% [38,39]. Regarding HCT recipients, treatment for HCV infection after HCT has been reported to be efficient in the specific population [40]. Recent progress in novel direct-acting antiviral agents might also be beneficial among HCV-positive recipients [41]. These anti-HCV therapies would play a critical role for the control and prevention of possible HCV-induced complications after HCT, as well as risk stratification by liver functional tests.

In summary, HCV seropositivity had an adverse impact on the clinical outcome following HCT, especially in the setting of unrelated HCT and in older patients. Careful evaluation before embarking on HCT and intensive assessment against complications are warranted in HCV-infected recipients. We may need to pay more attention to hematopoietic recovery and bacterial infections as well as hepatic problems in recipients with HCV. Based on these findings, a further prospective observation is warranted to overcome the adverse impact of HCV.

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Author Contributions

H.N. designed the study, analyzed data, and wrote the manuscript. S.K. and K.Y. gave advice regarding the methods, analyzed data, and wrote the manuscript. S.T., M.M., K.I., T.K., T.E., and K.M. collected data. H.S., Y.M., and T.N. collected data and were responsible for the management of data from JSHCT, JMDP, and JCBBN, respectively. R.S. analyzed and managed the unified registry database and wrote the manuscript. T.F. analyzed data, wrote the manuscript, and was responsible for the study and the Complication-WG of the JSHCT.

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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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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.

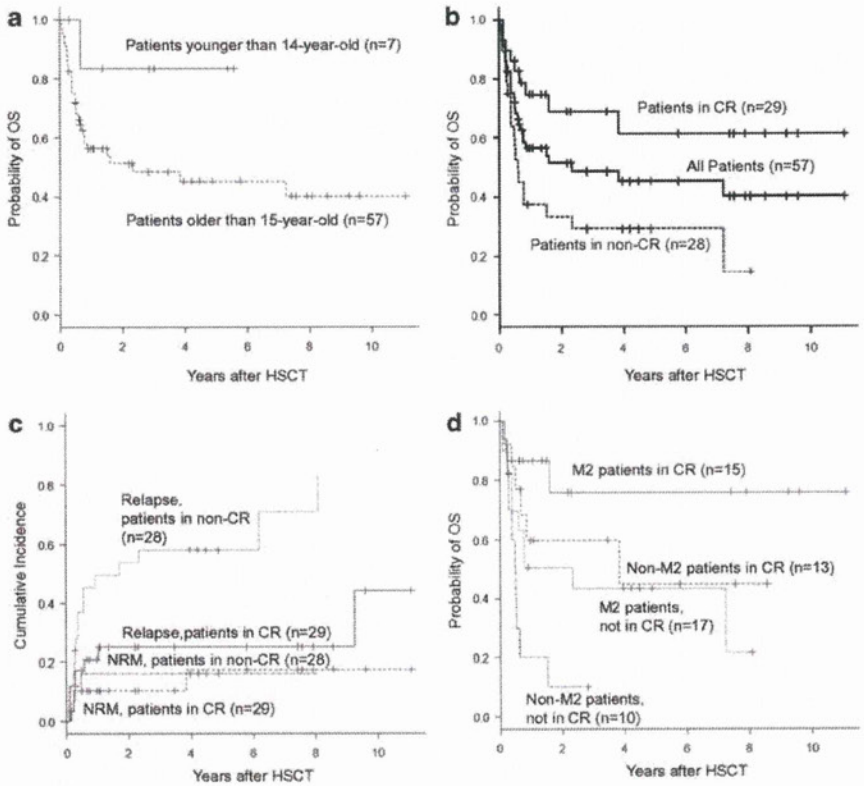

Figure 1. (**a**) The probabilities of OS in the patients with the t(6;9)(p23;q34) abnormality, stratified by age at HSCT. Solid line, pediatric patients younger than 14 years; dotted line, adult patients older than 15 years. (**b**) The probabilities of OS of the patients older than 15 years, stratified 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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ORIGINAL ARTICLE

Allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with t(6;9)(p23;q34) dramatically improves the patient prognosis: a matched-pair analysis

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Acute myeloid leukemia (AML) with t(6;9)(p23;q34) is well known to have a poor prognosis treated with chemotherapy and autotransplantation. The presence of this karyotype is an indicator for allogeneic hematopoietic stem cell transplantation (HSCT); however, the impact of t(6;9)(p23;q34) on the HSCT outcome remains unclear. We conducted a matched-pair analysis of *de novo* AML patients with and without t(6;9)(p23;q34) using data obtained from the Japanese HSCT data registry. A total of 57 patients with t(6;9)(p23;q34) received transplants between 1996 and 2007, and 171 of 2056 normal karyotype patients matched for age, disease status at HSCT and graft source were selected. The overall survival, disease-free survival, cumulative incidence of relapse and the non-relapse mortality in t(6;9)(p23;q34) patients were comparable to those for normal karyotype patients. A univariate analysis showed that t(6;9)(p23;q34) had no significant impact on the overall survival. These findings suggest that allogeneic HSCT may overcome the unfavorable impact of t(6;9)(p23;q34) as an independent prognostic factor.
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Introduction

Acute myeloid leukemia (AML) is a hematological malignancy resulting from the proliferation of leukemic stem cells. Because of the resistance of leukemic stem cells to chemotherapy,[1] long-term survival is generally seen in only 50% of patients treated with chemotherapy alone. Therefore, allogeneic stem cell transplantation (HSCT) is often considered as a curative treatment option.[2] AML is the most common indication for HSCT in North America and in Japan, but fatal transplant-related adverse events are difficult to avoid, despite the improvements in supportive treatment in recent years. Therefore, treatment of AML is hard to standardize, and the attending physician must make a decision on a case-by-case basis, weighing the advantages and disadvantages of HSCT.

The results of previous large clinical trials have indicated that abnormalities of the chromosomal karyotype are considered to be one of the most powerful factors to predict the patient prognosis.[3,4] AML with the unfavorable cytogenetic risk group, such as a partial deletion of the long arm of chromosome 7 (del (7q)), monosomy of chromosome 7 (−7) or with a complex karyotype is considered to be a good indication for HSCT, even during the first remission, because of the high cytogenetic risk associated with chemotherapy and the beneficial outcome that can be achieved by HSCT.[5–8]

The translocation of chromosome (6;9)(p23;q34) forming the *DEK/NUP214* fusion mRNA is observed in ~1% of AML cases.[9] The characteristics of AML with t(6;9)(p23;q34) are known to include development at a younger age,[10] resistance to chemotherapy and a very poor prognosis.[9] Therefore, the presence of this karyotype in AML patients is an indication for HSCT; however, the impact of t(6;9)(p23;q34) on the outcome of HSCT remains unclear because of the rarity of this entity. We conducted a retrospective study to examine the outcomes of HSCT in AML patients with t(6;9)(p23;q34) using the data from the Japan Society for Hematopoietic Cell Transplantation Data Registry.

Materials and methods

Study population

Clinical data were collected from the databases of the Japan Society for Hematopoietic Cell Transplantation and the Japan Cord Blood Bank Network using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after HSCT. Patients with *de novo* AML aged 15 years or older at the time of first HSCT and who received the transplant between January 1996 and December 2007 were extracted from the databases. We compared the clinical features and the outcomes among the patients with t(6;9)(p23;q34) and the patients with a normal karyotype in G-band staining. Cytogenetic data were analyzed according to the Southwestern Oncology Group criteria in each institution[7] instead of by central review. We selected patient pairs with t(6;9)(p23;q34)

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and the normal karyotype using an optimal matching method with the following three matching factors: recipient age, disease status at HSCT and graft source. This study was approved by the Committee for Nationwide Survey Data Management of the Japan Society for Hematopoietic Cell Transplantation. Written informed consent was obtained in accordance with the Declaration of Helsinki.

Statistical analysis

The overall survival (OS) was defined as the number of days from HSCT until death from any cause. Disease relapse was defined as the number of days from HSCT to relapse of the underlying disease. Non-relapse mortality was defined as death without relapse. Any patient who was alive at the last-follow-up date was censored. All statistical analyses were performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; http://www.r-project.org). Probabilities and times-to-events were compared between the two groups using the Mantel–Haenszel method and stratified Cox's proportional hazard modeling, respectively. The cumulative incidences of non-relapse mortality and relapse were calculated considering each other event as a competing risk, and were compared using the stratified Grey test.[11] *P* values were two sided, and outcomes were considered to be significant when $P \leqslant 0.05$.

Results

Patients' characteristics

A total of 2577 AML cases met the inclusion criteria. The number of cases with t(6;9)(p23;q34) and a normal karyotype was 57 and 2056, respectively; and 171 patients with the normal karyotype were selected for matched-pair analysis by a 1:3 matching ratio. The characteristics of the patients are shown in Table 1; there were no statistically significant differences between the t(6;9)(p23;q34) patients and the normal karyotype patients except the use of total body irradiation as a preconditioning regimen.

Survival, relapse and non-relapse mortality

The probability of OS in the patients with t(6;9)(p23;q34) was as good as that for patients with a normal karyotype (the probability of 5-year OS in t(6;9)(p23;q34) and normal karyotype patients was 45 and 40%, respectively; Figure 1a). When the t(6;9)(p23;q34) patients and the normal karyotype patients were further categorized according to the disease status at HSCT, the OS of the t(6;9)(p23;q34) patients and the normal karyotype patients were comparable in both the complete remission (CR) at HSCT patients and the non-CR at HSCT patients (Figure 1b). The probability of disease-free survival in these patients was also not significantly different (the probability of 5-year disease-free survival in patients with t(6;9)(p23;q34) and the normal karyotype was 42 and 33%, respectively; Figure 1c). The cumulative incidence of relapse (Figure 2a) and the non-relapse mortality (Figure 2b) in t(6;9)(p23;q34) patients were also comparable to those for normal karyotype patients (the 5-year cumulative incidence was 42% in t(6;9)(p23;q34) patients and 45% in normal karyotype patients for relapse ($P=0.34$) and 16 and 22% ($P=0.85$) for non-relapse mortality). The prognostic factors affecting OS revealed that there were no significant differences related to karyotype, gender, gender mismatch between donor and recipient, human leukocyte antigen disparity, recipient cytomegalovirus serostatus and use of total body irradiation for the preconditioning regimen by the univariate analyses (Table 2).

Table 1 Patient characteristics

	t(6;9)(p23;q34)	*Normal karyotype*	P-*value*
Age			
15–24	14	42	0.999
25–34	14	45	
35–44	20	58	
45–54	7	20	
55–64	2	6	
Gender			
Male	34	97	0.758
Female	23	74	
Disease status at HSCT			
CR1 or CR2	29	87	1.0
Not in remission	28	84	
Preconditioning regimen, TBI			
No	21	33	0.0102
Yes	33	131	
Donor			
Related	26	78	1.0
Unrelated bone marrow	18	54	
Unrelated cord blood	13	39	
Number of HLA mismatch			
0	24	47	0.379
1	5	23	
2	10	27	
3	0	2	

Abbreviations: CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; TBI, total body irradiation.

Discussion

Previous reports have confirmed the negative impact of t(6;9)(p23;q34) on the outcome after standard-dose chemotherapy and high-dose therapy with autologous stem cell transplantation in patients with AML.[9,10] The current matched-pair analysis of the nationwide survey demonstrated that the OS and the non-relapse mortality, as well as the relapse rate, were independent of the presence of t(6;9)(p23;q34) in allogeneic HSCT recipients, thus suggesting that allogeneic HSCT may be able to overcome the unfavorable effect of t(6;9)(p23;q34) in AML patients.

However, it is difficult to draw any firm conclusions regarding the results of the present analysis owing to the small number of patients in the matched-pairs subsets. These findings require confirmation in larger studies specifically in examining the impact of t(6;9)(p23;q34) status. Nevertheless, the suggestion that allogeneic HSCT appears to overcome the adverse survival impact of t(6;9)(p23;q34) is supported by other studies.[12,13] In a EBMT study of AML patients with t(6;9)(p23;q34), allogeneic HSCT produced responses that were independent of t(6;9)(p23;q34), and the 3-year OS of patients with t(6;9)(p23;q34) was as high as $51 \pm 7\%$, comparable to AML patients with the normal karyotype.[13] Also, the incidence of relapse following allogeneic HSCT appeared to be similar in patients with t(6;9)(p23;q34) compared with those without

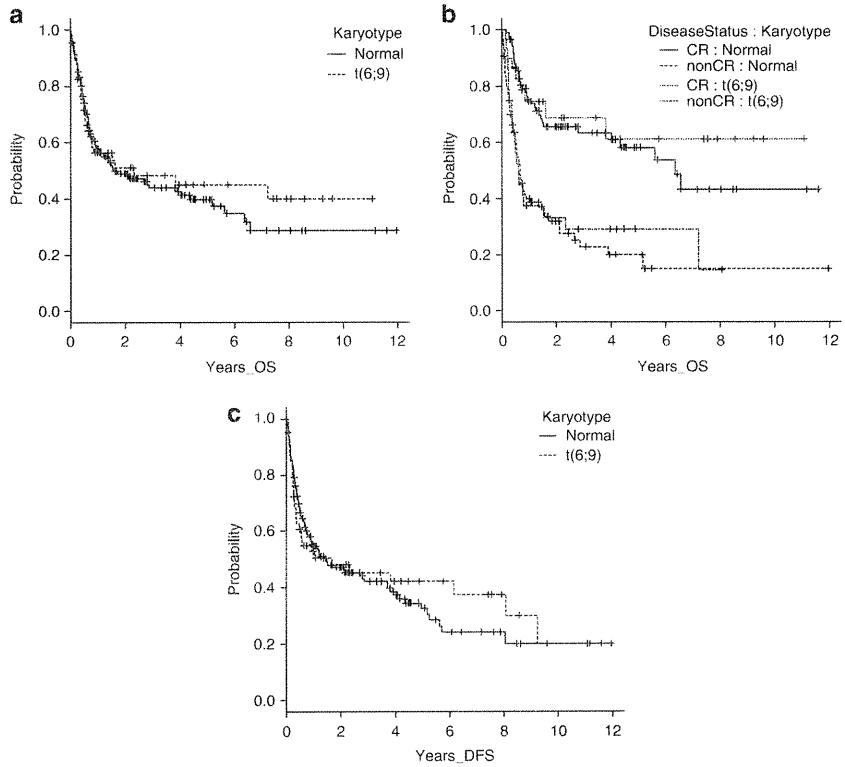

Figure 1 Survival of the patients. (**a**) The OS of the patients stratified by cytogenetics. Solid line, normal karyotype patients; dotted line, t(6;9) patients. (**b**) The OS of the patients grouped according to their disease status at transplantation. Solid line, normal karyotype patients in CR at HSCT; dashed line, normal karyotype patients in non-CR at HSCT; dotted line, t(6;9) patients in CR at HSCT; chain line, t(6;9) patients in non-CR at HSCT. (**c**) The disease-free survival of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

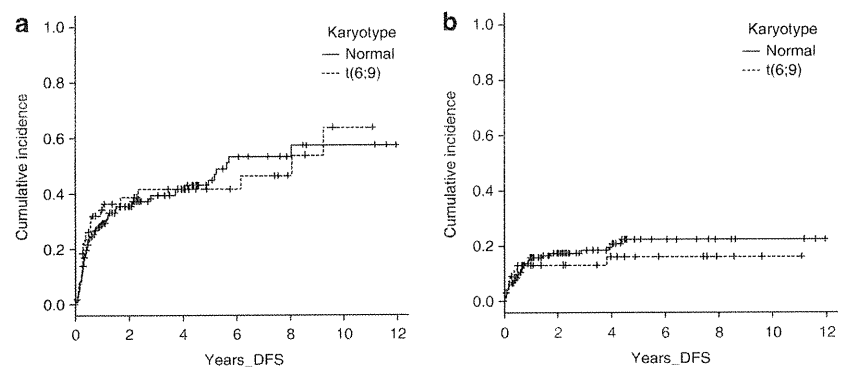

Figure 2 Cumulative incidence of events after transplantation stratified by cytogenetics. (**a**) The cumulative incidence of relapse of the patients. (**b**) The cumulative incidence of non-relapse mortality of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

Table 2 Prognostic factors affecting overall survival

	Risk factor	*Hazard ratio*	*95% CI*	P-*value*
Karyotype	t(6;9)	1.07	0.66–1.74	0.79
Gender	Male	1.06	0.64–1.73	0.83
Gender mismatch	Female to male	1.41	0.74–2.68	0.29
HLA compatibility	Mismatch	0.98	0.57–1.75	0.94
Recipient CMV	Positive	0.27	0.028–2.70	0.27
Donor CMV	Positive	1.51	0.61–3.78	0.37
TBI	Yes	1.47	0.75–2.90	0.26

Abbreviations: CMV, cytomegalovirus; HLA, human leukocyte antigen; TBI, total body irradiation.

t(6;9)(p23;q34). However, the EBMT study made it somewhat difficult to determine whether HSCT would lead to a good outcome, because 87% of the patients were transplanted while in CR, whereas only 29 of 57 (51%) patients in our study received HSCT in CR, which is a more clinically relevant expectation, as a CR is difficult to achieve in these patients.

In conclusion, the current study showed that AML patients with t(6;9)(p23;q34) can be expected to have a post-transplant survival comparable to patients with a normal karyotype, thereby supporting the opinion that they are good candidates for HSCT.

Conflict of interest

The authors declare no conflict of interest.

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Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia

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ABSTRACT

Recent advances in unrelated cord blood transplantation have increased chances and options available in allogeneic stem cell transplantation. The effect of HLA disparity on outcomes after cord blood transplantation was studied recently in mainly pediatric populations. Results showed that HLA matching in combination with total nucleated cell dose positively affects survival. The effect of HLA disparity after single-unit cord blood transplantation may be different in adults because their total nucleated cell dose is much lower compared to pediatric patients. We investigated the effect of HLA disparity on the outcome of single-unit unrelated cord blood transplantation separately in 498 children aged 15 years or under (HLA-A, HLA-B low-resolution, and HLA-DRB1 high-resolution matched [6/6], n=82, and one locus- [5/6], n=222, two loci- [4/6], n=158, three loci- [3/6] mismatched, n=36) and 1,880 adults (6/6, n=71; 5/6, n=309; 4/6, n=1,025; 3/6, n=475) with leukemia. With adjusted analyses, in children, 4/6 showed significantly increased risks of overall mortality (relative risk [RR]=1.61, P=0.042) and transplant-related mortality (RR=3.55, P=0.005) compared to 6/6. The risk of grade 2 to 4 acute GVHD was increased in 5/6 (RR=2.13, P=0.004) and 4/6 (RR=2.65, P<0.001). In adults, the risk of mortality did not increase with the number of mismatched loci (RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6). The risk of relapse was significantly decreased in 4/6 (RR=0.67, P=0.034). The risk of transplant-related mortality (TRM) or acute GVHD was not increased in 5/6 or 4/6. The effect of HLA disparity on transplant outcome differed between children and adults. In children, an increased number of mismatched HLA loci correlated with an increased risk of mortality. In adults, there was no increase in mortality with an increase in the number of mismatched HLA loci.

Introduction

Recent advances in unrelated cord blood transplantation (UCBT) have provided increased opportunities for patients with hematologic malignancies to receive hematopoietic stem cell transplantation (HSCT). This has led to an increased number of UCBT procedures over the past decade.[1,2] Clinical comparison studies of cord blood and bone marrow from unrelated donors have shown comparable results, which indicates that cord blood is a reasonable alternative donor / stem cell source.[3-12] These studies support the use of HLA-A, HLA-B, low-resolution and HLA-DRB1 zero- to two-loci-mismatched UCB for patients with leukemia in the absence of an HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched unrelated adult donor, and the use of UCB as a first-line option when a transplant is urgently required.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and HLA-A, HLA-B, HLA-C, and HLA-DRB1 allele matched bone marrow is currently the first alternative for HLA-identical sibling donors.[13-16] An increase in the number of HLA mismatches, antigen-level, or high-resolution, at HLA-A, HLA-B, HLA-C, or HLA-DRB1 loci from 8/8 to 7/8, or 7/8 to 6/8 was associated with higher mortality with an approximately 10% reduction in survival in UBM recipients.[12,13,15] Since HLA mismatches are better tolerated after UCB with a lower incidence of severe graft-*versus*-host disease (GVHD), up to two HLA antigen mismatches of HLA-A, HLA-B, low resolution and HLA-DRB1 high resolution are considered in the current CB selection algorithm. Several reports have recently described the effect of HLA disparity on the transplant outcomes after UCBT.[9,17,18] Eapen *et al.* reported the pos-

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sibility of a better outcome in HLA 6/6 matched UCB in 35 recipients, and Barker *et al.* confirmed these results with a larger number of UCB recipients.[9,18] However, these studies, which assessed the effect of HLA disparity on the outcome of single-unit CBT, were mainly conducted in pediatric populations in which the infused cell dose is much greater than that in adult recipients.

The aim of this study was to assess the effect of HLA disparity on the transplant outcomes after single-unit UCBT in pediatric and adult recipients. The accumulation of single-unit CBT in adult recipients has enabled us to assess separately the effect of HLA disparity on CBT outcomes in children and adults.

Design and Methods

Study design and data source

For this retrospective observational study, recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN). All 11 cord blood banks in Japan are affiliated with the JCBBN. JCBBN collected the recipients' clinical information at 100 days post-transplant through the Transplant Registry Unified Management Program (TRUMP) of the Japan Society of Hematopoietic Cell Transplantation (JSHCT).[19] Information on survival, disease status, and long-term complications including chronic graft-*versus*-host disease and second malignancies is renewed annually. Patient consent is not required for TRUMP registration of the JSHCT for the registry data consists of anonymized clinical information. This study was approved by the data management committees of the JSHCT and the JCBBN, and by the institutional review boards of Saitama Medical Center, Jichi Medical University and Nagoya University Graduate School of Medicine, Japan.

Patients

The subjects were patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), or myelodysplastic syndrome (MDS), who were recipients of their first UCBT between January 2000 and December 2009. Among 2,461 recipients of single-unit UCB with complete HLA-A, HLA-B, low-resolution and HLA-DRB1 high-resolution data, 51 recipients with 4 HLA mismatches were excluded. Thirty recipients who did not receive GVHD prophylaxis and 2 recipients for whom information regarding the conditioning regimen was missing were excluded. A total of 2378 single-unit UCB recipients (498 children aged 15 years or under at transplant, and 1880 adults aged 16 years or over at transplant) were subjects for analysis.

HLA typing

Histocompatibility data for low-resolution typing for the HLA-A, HLA-B, and HLA-DR loci and high-resolution typing for HLA-DRB1 were obtained from the TRUMP database which includes HLA information provided by cord blood banks or transplant centers. The level of HLA typing in the present study was HLA-A, HLA-B, low-resolution, and HLA-DRB1 high-resolution, as in other studies in Europe and North America. However, according to current practice in Japan, mismatches in HLA-DR loci were counted at the low-resolution level at UCB unit selection. Therefore, results regarding the effect of HLA mismatches in HLA-A, HLA-B, and HLA-DR low-resolution are also provided (*Online Supplementary Table S1*). Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients compared to HLA class I mismatched recipients. Thus, in Japan, a single-DRB1-mismatched UBM donor is preferred over a single-A-mismatched UBM or single-B-mismatched UBM donor.[15,20] This background affected HLA typing strategy of HLA-DR low-resolution typing instead of high-resolution typing for selection of cord blood units in Japan. This observation may explain the fact that the frequency of 4/6 grafts is higher in this cohort than in cohorts in Europe and the USA.

Definitions

The primary outcome of the analyses was overall survival, defined as time from transplant to death from any cause. Several secondary end points were also analyzed. Neutrophil recovery was defined as an absolute neutrophil count of at least 0.5×10^9/L cells per cubic millimeter for three consecutive points; platelet recovery was defined as a count of at least 50×10^9 platelets per cubic millimeter without transfusion support. The recipients of reduced-intensity conditioning were also defined with the criteria above, according to the previous report that confirmed complete donor chimeras of all engrafted patients after CBT with reduced-intensity conditioning.[21] Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[22,23] Relapse was defined as the recurrence of underlying hematologic malignant diseases. Transplant-related death was defined as death during a continuous remission.

Statistical analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages are shown for categorical variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and transplant-related mortality (TRM).[24] Gray's test was used for group comparisons of cumulative incidences.[25] An adjusted comparison of the groups with regard to overall survival (OS) was performed with the use of the Cox's proportional-hazards regression model.[26] For other outcomes with competing risks, Fine and Gray's proportional-hazards model for the subdistribution of a competing risk was used.[27] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event. For GVHD, death without GVHD and relapse were competing events. For relapse, death without relapse was the competing event, and for transplant-related mortality (TRM), relapse was the competing event.[28] For acute GVHD, subjects were limited to those who engrafted, and for chronic GVHD, subjects were limited to those who engrafted and survived at least 100 days after transplantation.

The variables considered were the patient's age at transplant (5 years or over *vs.* under 5 years for pediatric recipients, and 50 years or over *vs.* under 50 years for adult recipients; cut-off points were around the median in each group), patient's sex, donor-patient sex mismatch (matched *vs.* male to female *vs.* female to male), donor-patient ABO mismatch (major mismatch *vs.* matched or minor mismatch), diagnosis (AML, ALL, CML or MDS), disease status at conditioning (first or second complete remission (CR) of AML, 1CR of ALL, first chronic phase of CML, and refractory anemia or refractory anemia with ringed sideroblasts as standard-risk diseases *vs.* advanced for all others), the conditioning regimen (reduced-intensity conditioning *vs.* myeloablative conditioning), and the type of prophylaxis against GVHD (tacrolimus-based *vs.* cyclosporine-based). Conditioning regimens were classified as myeloablative if total-body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was used based on the report from the Center for International Blood and Marrow Transplant Research.[29,30] We cat-

egorized patients for whom there was insufficient information regarding the doses of agents or radiation used for the conditioning regimen according to information on the conditioning intensity (i.e. whether or not the conditioning regimen was intended to be myeloablative) as reported by the treating clinicians. The cryopreserved total nucleated cell dose was categorized as >10.0x10⁷/kg, 5.0-9.9 x 10⁷/kg, 2.5-4.9x10⁷/kg, or <2.5 x 10⁷/kg for children, and >3.0x10⁷/kg, 2.5-2.9x10⁷/kg, 2.0-2.4x10⁷/kg, or <2.0x10⁷/kg for adults. HLA disparity and nucleated cell dose were maintained in the model. Since patient age was highly correlated with the total nucleated cell dose in children, age was excluded from multivariate analyses for pediatric recipients. Other variables were selected in a backward stepwise manner with a variable retention criterion of $P<0.05$. Interaction between HLA disparity and adult (patient age at transplant 16 years or over) or child (patient age at transplant 15 years or under) was tested for overall survival by using a Cox's proportional-hazards regression model adjusted by other significant covariates in the final model for adult and pediatric recipients except for patient age. All P values were two-sided.

Results

Patients' characteristics

Table 1 shows patients' characteristics, their disease, and transplant regimens. Median age at transplant was five years (range 0-15) in 498 pediatric and 49 years (range 16-82) in 1880 adult recipients of single-unit CBT. The proportion of females was 45% in both children and adults. Among children, the proportion of patients with ALL was greatest (58%) followed by that of patients with AML (34%). Among adults, the most frequent disease was AML (59%), followed by ALL (22%) and MDS (13%). The median number of cryopreserved total nucleated cells received in children was 5.30 x 10⁷/kg, which was significantly greater (approximately double) than the number of nucleated cells received in adult patients (2.52 x 10⁷/kg). In adults, only 33 patients (2%) received CB with a total nucleated cell dose greater than or equal to 5.0 x 10⁷/kg. In children, 82 patients (16%) received HLA-matched (6/6) UCB, 222 (45%) received one-locus-mismatched (5/6), 158 (32%) received two-loci-mismatched (4/6), and 36 (7%) received three-loci-mismatched (3/6) UCB. For adults, the numbers and proportions of recipients were 71 (4%) for 6/6, 309 (16%) for 5/6, 1025 (55%) for 4/6, and 475 (25%) for 3/6. Among those who received 3/6 UCB, only 2 pediatric and 11 adult patients received three HLA-A, HLA-B, HLA-DR low-resolution mismatched UCB. Eighty-eight percent (TBI regimen 62%, non-TBI regimen 26%) and 62% (TBI regimen 56%, non-TBI regimen 6%) of children and adults, respectively, received myeloablative conditioning. Fludarabine-based reduced-intensity conditioning was given to 34% of adult recipients. T-cell depletion *in vivo* with antithymocyte globulin or antilymphocyte globulin was performed in only 6 (2%) child recipients and 26 (1%) adult recipients. The median follow-up period for survivors was 2.4 years (range 0.1-9.5) for pediatric recipients and 2.1 (range 0.1-9.0) years for adult recipients.

Outcome

Overall survival, relapse, and transplant-related mortality: among children, overall mortality in 4/6 UCB recipients was significantly higher than that in 6/6 UCB recipients (RR=1.61, 95% confidence interval [CI], 1.02-2.56, P=0.042) (Table 2). Overall mortality increased with the number of mismatched loci in children (P for trend 0.043). The increased mortality in 4/6 UCB recipients was mainly affected by increased transplant-related mortality (TRM) (RR=3.55, 95%CI: 1.47-8.58, P=0.005) (P for trend 0.002) but not by the risk of relapse (RR=0.77, 95%CI: 0.48-1.24, P=0.392) in children. Among children, there were no differences in the risks of mortality and relapse between 5/6 UCB recipients (RR=1.07, P=0.765 for overall mortality; RR=1.06, P=0.794 for relapse; and RR=1.29, P=0.58 for TRM) and 6/6 UCB recipients (Table 2).

In adults, the number of HLA mismatches was not significantly associated with increased mortality (for overall mortality: RR=0.99, P=0.944 for 5/6; RR=0.88, P=0.436 for 4/6; RR=0.95, P=0.751 for 3/6; for TRM, RR=1.41, P=0.205 for 5/6; RR=1.24, P=0.408 for 4/6; RR=1.29, P=0.339 for 3/6). A two-loci mismatch was associated with a decreased risk of relapse in adult recipients (RR=0.70, P=0.075 for 5/6; RR=0.67, P=0.034 for 4/6; RR=0.70, P=0.07 for 3/6) (Table 2). The risks of mortality were similar when subjects were limited to those with standard risk disease status or to those with advanced risk disease status at transplant, to those who received myeloablative conditioning or to those who received reduced-intensity conditioning (*Online Supplementary Table S2*). A decreased risk of relapse was more prominent in patients with acute myeloid leukemia, and those who received reduced-intensity conditioning (*Online Supplementary Table S2*).)

Figure 1 shows unadjusted overall survival curves in children and adults. In children, the unadjusted probabilities of survival at three years post-transplant were 66% for 6/6, 62% for 5/6, 45% for 4/6, and 62% for 3/6 (P=0.032) (Figure 1A). In adults, the survival probabilities in all of the HLA disparity groups were similar (38% for 6/6, 37% for 5/6, 39% for 4/6, and 40% for 3/6 at three years post-transplant, P=0.567) (Figure 1B). A similar trend was seen when subjects were limited to standard-risk disease status at transplant (81% for 6/6, 76% for 5/6, 57% for 4/6, and 81% for 3/6 at three years post-transplant, P=0.035, for children; 51% for 6/6, 57% for 5/6, 58% for 4/6, and 55% for 3/6 at three years post-transplant, P=0.375, for adults) (*Online Supplementary Figure S1*).

A test of the interaction between HLA disparity and age (adult *vs.* child) revealed that the effect of HLA disparity on overall survival differed significantly between the pediatric and adult patient groups (P=0.009 for HLA disparity of 0-1 mismatches *vs.* 2-3 mismatches).

Hematologic recovery

The cryopreserved total nucleated cell dose significantly affected neutrophil and platelet recovery in children and neutrophil recovery in adults (Table 3). HLA disparity did not significantly affect neutrophil or platelet recovery in adults or children for neutrophil recovery: RR=1.03, P=0.823 for 5/6; RR=0.96, P=0.799 for 4/6; RR=0.67, P=0.068 for 3/6 in children; RR=0.89, P=0.436 for 5/6; RR=0.92, P=0.576 for 4/6; RR=0.84, P=0.243 for 3/6 in adults; for platelet recovery: RR=0.89, P=0.438 for 5/6; RR=0.75, P=0.09 for 4/6; RR=0.71, P=0.164 for 3/6 in children; RR=1.05, P=0.775 for 5/6; RR=1.05, P=0.791 for 4/6; RR=0.99, P=0.951 in 3/6 in adults (Table 3).

Table 1. Patients', disease, and transplant characteristics of pediatric and adult recipients of single-unit cord blood.

Characteristics	Children (age<16) N.	(%)	Adult (age>16) N.	(%)
N. of transplants	498		1880	
Patient age at transplant				
Median (range)	5 (0-15)		49 (16-82)	
0-9 years	378	(76)		
10-19 years	120	(24)	88	(5)
20-29 years			236	(13)
30-39 years			317	(17)
40-49 years			351	(19)
50-59 years			492	(26)
≥60 years or older			396	(21)
Patient sex				
Male	275	(55)	1039	(55)
Female	223	(45)	841	(45)
Sex matching				
Matched	207	(42)	696	(37)
Male to female	114	(23)	391	(21)
Female to male	125	(25)	485	(26)
Unknown	52	(10)	308	(16)
Diagnosis				
AML	170	(34)	1115	(59)
ALL	290	(58)	418	(22)
CML	7	(1)	106	(6)
MDS	31	(6)	241	(13)
Disease status				
Standard	247	(50)	673	(36)
Advanced	236	(47)	1127	(60)
Unknown	15	(3)	80	(4)
ABO matching				
Matched	182	(37)	602	(32)
Minor mismatch	127	(26)	522	(28)
Major mismatch	113	(23)	451	(24)
Bidirectional	75	(15)	301	(16)
Unknown	1	(<1)	4	(<1)
HLA mismatched number				
Matched (6/6)	82	(16)	71	(4)
One locus mismatched (5/6)	222	(45)	309	(16)
Two loci mismatched (4/6)	158	(32)	1025	(55)
Three loci mismatched (3/6)	36	(7)	475	(25)
N. of cryopreserved nucleated cells (x10⁷/kg)				
Median	5.30		2.52	
Range	0.81-38.7		0.71-9.98	
N. of cryopreserved CD34-positive cells (x10⁵/kg)				
Median	1.68		0.83	
Range	0.072-65.66		0.07-14.02	
Preparative regimen*				
MAST				
CY+TBI	216	(43)	891	(47)
Other TBI regimen	93	(19)	162	(9)
BU+CY	86	(17)	65	(3)
Other non-TBI regimen	41	(8)	47	(3)
RIST				
FL+BU+other	6	(1)	172	(9)
FL+CY+other	12	(2)	119	(6)
FL+Mel+other	21	(4)	357	(19)
Other RIST	23	(5)	67	(4)
T-cell depletion *in vivo***				
ATG or ALG use	9	(2)	26	(1)
GVHD prophylaxis***				
Cyclosporine A + sMTX	157	(32)	748	(40)
Cyclosporine A + MMF/steroid	37	(7)	99	(5)
Cyclosporine A alone	31	(6)	142	(8)
Tacrolimus + sMTX	216	(43)	434	(23)
Tacrolimus + MMF/steroid	24	(5)	132	(7)
Tacrolimus alone	20	(4)	304	(16)
Others	13	(3)	21	(1)

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*CY: cyclophosphamide; CA: citarabine; BU: busulfan; TBI: total body irradiation; FL: fludarabine; Mel: melphalan, **ATG: antithymocyte globulin; ALG: antilymphocyte globulin; ***sMTX: short-term methotrexate; MMF: mycophenolate mofetil.

Acute and chronic graft-versus-host disease

The risk of grade 2 to 4 acute GVHD was significantly higher in HLA-mismatched UCB pediatric recipients (RR=2.13, P=0.004 for 5/6; RR=2.65, P<0.001 for 4/6; RR=2.39, P=0.0015 for 3/6; P for trend 0.001) (Table 4). The risk of chronic GVHD and extensive-type chronic GVHD was also significantly higher in 4/6 UCB recipients (RR=2.99, P=0.005 for chronic GVHD, and RR=7.62, P=0.047 for extensive-type chronic GVHD), and the risks increased according to the number of mismatches (P for trend, 0.002 for chronic GVHD, 0.005 for extensive-type chronic GVHD). In adults, in contrast to the results in children, there were no differences in the risks of grade 2 to 4 acute GVHD in 5/6 and 4/6 UCB recipients (for grade 2 to 4 acute GVHD, RR=1.03, P=0.916 for 5/6, RR=1.27, P=0.276 for 4/6). The risk of grade 2 to 4 acute GVHD was higher for 3/6 (RR=1.72, P=0.017). In adult recipients, the risk of chronic GVHD was increased in recipients of 4/6 UCB (RR=1.90, P=0.04), however, there were no differences in the risk of extensive-type chronic GVHD (RR=1.15, P=0.758 for 5/6; RR=1.62, P=0.253 for 4/6; RR=1.28, P=0.574 for 3/6) (Table 4).

Effect of total nucleated cell dose on outcome

An increase in the cryopreserved total nucleated cell dose increased the incidence of neutrophil recovery in both children and adults, as well as the incidence of platelet recovery in children (Table 3). The cumulative incidences of neutrophil recovery were 94% for >10 x 10^7/kg, 88% for 5.0-9.9 x 10^7/kg, 82% for 2.5-4.9 x 10^7/kg, and 86% for <2.5 x 10^7/kg in children (P<0.001) (Figure 2A). The cell dose was significantly correlated with the recipient's age at transplant in children (the median ages were one year for >10 x 10^7/kg, 3 years for 5.0-9.9 x 10^7/kg, 8 years for 2.5-4.9 x 10^7/kg, and 12 years for <2.5 x 10^7/kg). The cumulative incidences of neutrophil recovery were 76% for >2.5 x 10^7/kg and 74% for <2.5 x 10^7/kg in adults (P=0.007) (Figure 2B). The cumulative incidences of TRM at three years post-transplant were 13% for >10 x 10^7/kg, 14% for 5.0-9.9 x 10^7/kg, 14% for 2.5-4.9 x 10^7/kg, and 14% for <2.5x10^7/kg in children (P=0.98) and 29% for >2.5x10^7/kg and 28% for <2.5 x 10^7/kg in adults (P=0.77) (*Online Supplementary Figure S2*). The probabilities of overall survival at three years post-transplant were 68% for >10x10^7/kg, 53% for 5.0-9.9 x 10^7/kg, 57% for 2.5-4.9 x 10^7/kg, and 55% for <2.5x10^7/kg in children (P=0.30) and 36% for >2.5 x 107/kg and 41% for <2.5x10^7/kg in adults (P=0.13). A lower total nucleated cell dose was neither associated with increased mortality in children or adults in multivariate analyses (Table 2). Thus, there was no combined effect of HLA disparity and total nucleated cell dose on mortality neither in children nor in adults (cumulative

incidence of TRM at three years post-transplant, 8% for 6/6, 11% for 5/6 and >5 x 10^7/kg, 11% for 5/6 and 2.5-4.9 x 10^7/kg, 0% for 5/6 and <2.5 x 10^7/kg, 23% for 4/6 and >5 x 10^7/kg, 24% for 4/6 and 2.5-4.9 x 10^7/kg, 25% for 4/6 and <2.5 x 10^7/kg in children, and 23% for 6/6, 29% for 5/6 and >2.5 x 10^7/kg, 30% for 5/6 and <2.5 x 10^7/kg, 27% for 4/6 and >2.5 x 10^7/kg, 27% for 4/6 and <2.5 x 10^7/kg in adults (*Online Supplementary Figure S3*).

Association of outcomes with the type of HLA mismatches for 4/6 adult recipients

The large number of adult recipients of 4/6 CB enabled us to analyze association of outcomes with the type of HLA mismatches in this population. The number of recipients were 7 for HLA-A double mismatch, 170 for HLA-A and HLA-B mismatch, 190 for HLA-A and HLA-DRB1 mismatch, 36 for HLA-B double mismatch, 581 for HLA-B and HLA-DRB1 mismatch, and 41 for HLA-DRB1 double mismatch. With adjusted analyses, adjusted with same variables in the final model of all adult recipients, there was no significant effect of HLA mismatch types on overall mortality with HLA-A and HLA-B mismatch as the reference (*Online Supplementary Table S3*). The risk of relapse was significantly decreased in HLA-A and HLA-DRB1

Table 2. Multivariate analyses of overall survival, relapse, and transplant-related mortality.

		Overall mortality			Relapse			Transplant-related mortality		
Outcome	N.	RR	95%CI	P	RR	95%CI	P	RR	95%CI	P
Children 15 years or younger										
HLA disparity										
Matched (6/6)	82	1.00			1.00			1.00		
5/6	222	1.07	(0.68-1.69)	0.765	1.06	(0.68-1.65)	0.794	1.29	(0.52-3.23)	0.58
4/6	158	1.61	(1.02-2.56)	0.042	0.77	(0.48-1.24)	0.282	3.55	(1.47-8.58)	0.005
3/6	36	1.25	(0.65-2.42)	0.498	0.91	(0.45-1.86)	0.802	1.56	(0.43-5.63)	0.497
Total nucleated cell dose										
≥10.0x10^7/kg	85	1.00			1.00			1.00		
5.0-9.9x10^7/kg	169	1.14	(0.72-1.79)	0.579	1.10	(0.69-1.75)	0.684	0.82	(0.40-1.68)	0.592
2.5-4.9x10^7/kg	190	0.92	(0.58-1.45)	0.707	0.90	(0.56-1.44)	0.651	0.90	(0.45-1.80)	0.77
<2.5x10^7/kg	43	0.88	(0.47-1.67)	0.701	0.98	(0.53-1.83)	0.961	0.67	(0.24-1.88)	0.443
Adults 16 years or older										
HLA disparity										
Matched (6/6)	71	1.00			1.00			1.00		
5/6	309	0.99	(0.71-1.38)	0.944	0.70	(0.47-1.04)	0.075	1.41	(0.83-2.41)	0.205
4/6	1025	0.88	(0.65-1.21)	0.436	0.67	(0.47-0.97)	0.034	1.24	(0.75-2.04)	0.408
3/6	475	0.95	(0.69-1.31)	0.751	0.70	(0.48-1.03)	0.07	1.29	(0.77-2.16)	0.339
Total nucleated cell dose										
≥3.0x10^7/kg	439	1.00			1.00			1.00		
2.5-2.9x10^7/kg	492	0.99	(0.83-1.17)	0.876	0.86	(0.70-1.06)	0.167	1.10	(0.86-1.42)	0.445
2.0-2.4x10^7/kg	705	0.86	(0.72-1.01)	0.06	0.79	(0.65-0.97)	0.021	1.05	(0.83-1.33)	0.694
<2.0x10^7/kg	183	0.93	(0.73-1.18)	0.562	0.79	(0.59-1.07)	0.126	1.00	(0.70-1.45)	0.983

For overall mortality, other predictive variables were advanced disease status at transplant in children, and age at transplant over 50 years, male sex, advanced disease status at transplant, chronic myeloid leukemia (associated with a lower risk of mortality), and reduced-intensity conditioning in adults. For relapse, other predictive variables were advanced disease status at transplant, and acute lymphoblastic leukemia or myelodysplastic syndrome (associated with a lower risk of relapse) in children, and advanced disease status at transplant and myelodysplastic syndrome (associated with a lower risk of relapse) in adults. For transplant-related mortality, there was no other predictive variable in children. Other predictive variables for adults were age at transplant over 50 years and female to male donor-recipient sex mismatch.

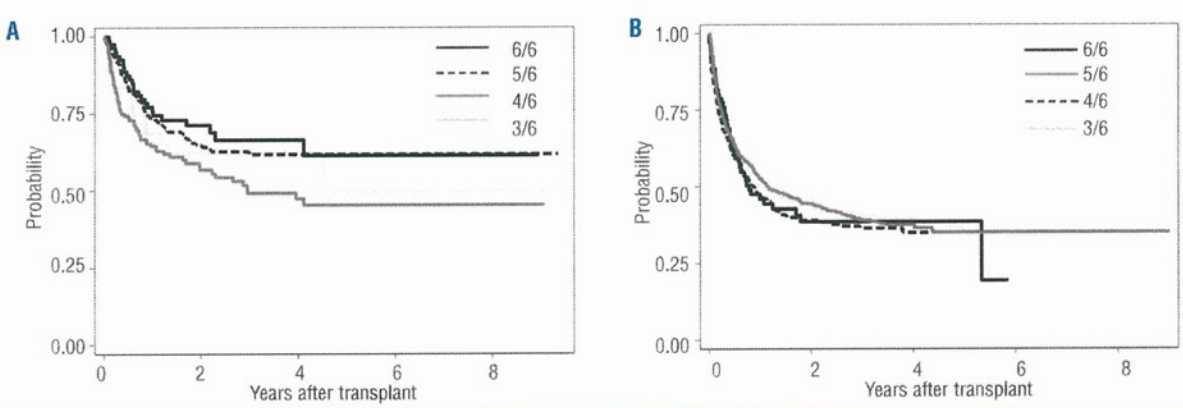

Figure 1. Unadjusted probabilities of overall survival in HLA disparity groups for pediatric (A) and adult (B) recipients with leukemia. (A) In children, the unadjusted probabilities of survival at three years post-transplant were 66% for recipients of HLA matched (6/6), 62% for one-locus-mismatched (5/6), 45% for two-loci-mismatched (4/6), and 62% for three-loci-mismatched (3/6) single-unit unrelated cord blood (P=0.032). (B) In adults, these probabilities were 38% 37%, 39%, and 40% respectively (P=0.567) (B).

mismatch, HLA-B and HLA-DRB1 mismatch, and HLA-DRB1 double mismatch recipients (RR=0.70, *P*=0.045; RR=0.76, *P*=0.047; and RR=0.46, *P*=0.03, respectively). The risk of transplant-related mortality was significantly increased in HLA-DRB1 double mismatch recipients (RR=2.06, *P*=0.025). There was no significant effect of HLA mismatch types for risks of grade 2 to 4 and grade 3 to 4 acute GVHD (*Online Supplementary Table S3*).

Discussion

Our main objective was to assess the effect of HLA disparity on survival after single-unit UCBT in children and adults, and to obtain data that could be useful for the selection of an appropriate cord blood unit for patients with leukemia. Our study is the first to assess the effect of UCB HLA-matching on the transplant outcome in a large

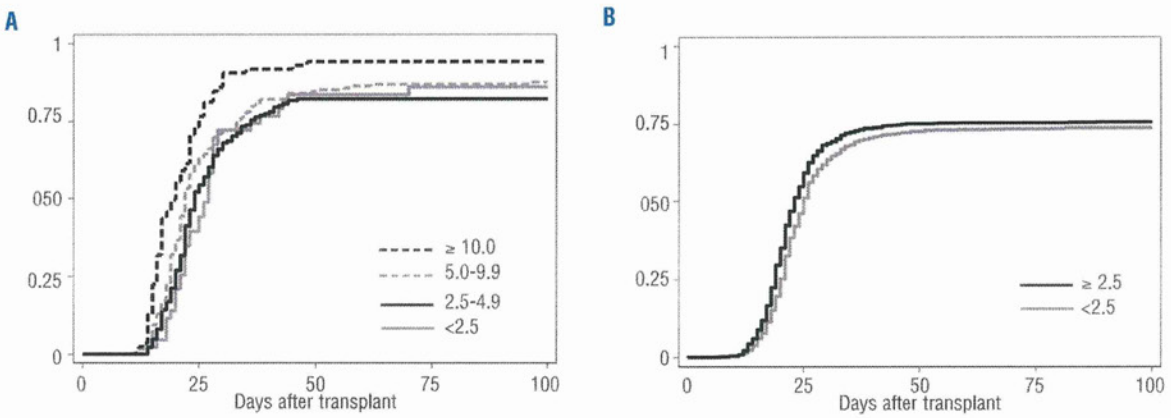

Figure 2. Unadjusted cumulative incidences of neutrophil recovery in total nucleated cell dose groups for pediatric (A) and adult (B) recipients with leukemia. (A) In children, the unadjusted cumulative incidences of neutrophil recovery were 94% for >10x10^7/kg, 88% for 5.0-9.9 x10^7/kg, 82% for 2.5-4.9x10^7/kg, and 86% for <2.5x10^7/kg (*P*<0.001). (B) In adults, these incidences were 76% for >2.5x10^7/kg and 74% for <2.5 x10^7/kg (*P*=0.007).

Table 3. Multivariate analyses of neutrophil and platelet recovery.

	Children 15 ≤years or younger					Adults ≥16 years or older			
Outcome	N	RR	95%CI	*P* value		N	RR	95%CI	*P*
Neutrophil recovery									
HLA disparity									
Matched (6/6)	82	1.00				71	1.00		
5/6	222	1.03	(0.77-1.39)	0.823		309	0.89	(0.66-1.19)	0.436
4/6	158	0.96	(0.71-1.30)	0.799		1025	0.92	(0.70-1.22)	0.576
3/6	36	0.67	(0.44-1.03)	0.068		475	0.84	(0.64-1.12)	0.243
Total nucleated cell dose									
≥>10.0x10^7/kg	85	1.00			≥3.0x10^7/kg	439	1.00		
5.0-9.9x10^7/kg	169	0.66	(0.49-0.89)	0.007	2.5-2.9x10^7/kg	492	0.84	(0.72-0.97)	0.021
2.5-4.9x10^7/kg	190	0.50	(0.37-0.67)	<0.001	2.0-2.4x10^7/kg	705	0.79	(0.68-0.90)	0.001
<2.5x10^7/kg	43	0.54	(0.38-0.77)	0.001	<2.0x10^7/kg	183	0.78	(0.64-0.94)	0.009
Platelet recovery									
HLA disparity									
Matched (6/6)	82	1.00				71	1.00		
5/6	222	0.89	(0.66-1.20)	0.438		309	1.05	(0.73-1.52)	0.775
4/6	158	0.75	(0.54-1.05)	0.09		1025	1.05	(0.74-1.48)	0.791
3/6	36	0.71	(0.44-1.15)	0.164		475	0.99	(0.69-1.41)	0.951
Total nucleated cell dose									
≥10.0x10^7/kg	85	1.00			≥3.0x10^7/kg	439	1.00		
5.0-9.9x10^7/kg	169	0.93	(0.68-1.29)	0.681	2.5-2.9x10^7/kg	492	0.84	(0.70-1.01)	0.058
2.5-4.9x10^7/kg	190	0.70	(0.51-0.97)	0.03	2.0-2.4x10^7/kg	705	0.86	(0.73-1.02)	0.078
<2.5x10^7/kg	43	0.70	(0.45-1.07)	0.101	<2.0x10^7/kg	183	0.72	(0.57-0.91)	0.007

For neutrophil recovery, other predictive variables were acute lymphoblastic leukemia in children (with a higher neutrophil recovery), and advanced disease status at transplant in adults. For platelet recovery, other predictive variables were advanced disease status at transplant in children, and age at transplant over 50 years, male sex, and advanced disease status at transplant in adults.