Table 2. Effect of HLA locus matching on acute GVHD and chronic GVHD in a multivariable competing risk regression model

			Acute GVHD (Grade III-IV)†			Acute GVHD (Grade II-IV)†				Chronic GVHD‡		
HLA	Match or mismatch*	N	RR	95% CI	P	RR	95% CI	P	N	RR	95% CI	P
A	Match	7048	1.00		.001	1.00		.002	5892	1.00		.328
	Mismatch	850	1.29	1.10-1.51		1.18	1.06-1.32		636	1.06	0.94-1.21	
B	Match	7475	1.00		.001	1.00		.001	6217	1.00		.235
	Mismatch	423	1.42	1.16-1.73		1.28	1.11-1.48		311	1.10	0.94-1.30	
C	Match	5565	1.00		<.001	1.00		<.001	4716	1.00		<.001
	Mismatch	2333	1.63	1.45-1.83		1.27	1.17-1.37		1812	1.24	1.13-1.35	
DRB1	Match	5878	1.00		.022	1.00		<.001	4936	1.00		.262
	Mismatch	2020	1.21	1.03-1.43		1.24	1.11-1.39		1592	0.93	0.82-1.05	
DQB1	Match	5681	1.00		.336	1.00		.126	4758	1.00		.018
	Mismatch	2217	1.08	0.92-1.27		1.09	0.98-1.22		1770	1.15	1.03-1.30	
DPB1	Match	2604	1.00		.001	1.00		<.001	2223	1.00		.367
	Mismatch	5294	1.23	1.09-1.38		1.36	1.26-1.47		4305	1.04	0.96-1.12	

RR of respective HLA locus mismatches at the allele level was compared with HLA match adjusted with other HLA locus matching and clinical factors as listed in Table 1.
CI, confidence interval.
*GVH direction.
†Survived 7 or more days.
‡Survived 100 or more days.

Statistical analysis

Cumulative incidence of acute GVHD was assessed by a method described elsewhere.[22] Overall survival was calculated using the Kaplan-Meier method. Competing events were defined as death without acute GVHD for acute GVHD; death without chronic GVHD for chronic GVHD; death without neutrophil engraftment for neutrophil engraftment; and death without relapse for leukemia relapse. Multivariable competing risk regression analyses[23,24] were conducted to evaluate the impact of acute GVHD, chronic GVHD, leukemia relapse and neutrophil engraftment, and a Cox proportional regression model was used to evaluate the impact of mortality. The relative risk (RR) of HLA locus mismatch was compared with HLA locus match in the GVH direction for acute GVHD, chronic GVHD, leukemia relapse and mortality, and in the HVG direction for neutrophil engraftment. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), disease, risk of leukemia relapse (standard and high), GVHD prophylaxis (cyclosporine-based regimen, tacrolimus-based regimen, and other regimen without cyclosporine and tacrolimus), preconditioning (myeloablative and reduced intensity), and period of transplant year (1992-2000, 2001-2005, 2006-2010). Transplanted cell number and ABO blood type matching were added as confounders in analyses of neutrophil engraftment. Missing data for confounder variables were treated as an unknown group. Acute GVHD, leukemia relapse, neutrophil engraftment, and survival were assessed in patients who survived >7 days, and chronic GVHD at 2 years was assessed in patients who survived 100 or more days after transplantation. Leukemia relapse at 5 years was assessed in patients who survived >7 days after transplantation for leukemia with AML, ALL, and CML. Risk of chronic GVHD on leukemia relapse was assessed by time-dependent covariate analysis in leukemia patients who survived 100 or more days after transplantation. Neutrophil engraftment at 100 days was assessed in all patients. A P value of <.01 was considered significant. All analyses were conducted using STATA version 12 (Stata Corp).

Results

Effect of HLA locus matching on acute GVHD and chronic GVHD

RR of HLA allele mismatch compared with HLA allele match for grade III-IV acute GVHD was highly significant for HLA-A, -B, -C, and -DPB1 (RR 1.29, $P = .001$; 1.42, $P = .001$; 1.63, $P < .001$; and 1.23, $P = .001$, respectively), but was not significant for HLA-DRB1 or -DQB1 (Table 2). RR of grade II-IV acute GVHD was highly significant for HLA-A, -B, -C, -DRB1, and -DPB1 (RR 1.18, $P = .002$; 1.28, $P = .001$; 1.27, $P < .001$; 1.24, $P < .001$; and 1.36, $P < .001$, respectively), but was not significant for HLA-DQB1 (Table 2).

RR of HLA allele mismatch compared with HLA allele match for chronic GVHD was significant for HLA-C (RR 1.24 $P < .001$), but not significant for HLA-A, -B, -DRB1, -DQB1, or -DPB1 (Table 2).

Effect of HLA locus matching on survival

RR of HLA allele mismatch compared with HLA allele match for mortality was highly significant in the HLA class I locus, namely HLA-A (1.29, $P < .001$), HLA-B (1.27, $P < .001$) and HLA-C (1.21, $P < .001$), but was not significant in the HLA class II locus, namely HLA-DRB1, -DQB1, and -DPB1 (Table 3).

Positive interaction of HLA-DRB1 mismatch and HLA-DQB1 mismatch in the risk of acute GVHD and survival

As HLA-DRB1 and HLA-DQB1 matching are closely linked in the HLA region and matching probability for HLA-DRB1 and HLA-DQB1 was 89%, stratified analysis of HLA-DRB1 matching and HLA-DQB1 matching was performed (Table 4). Pairs with HLA-DRB1 and HLA-DQB1 double (DRB1_DQB1) mismatch showed a significant risk of acute GVHD compared with pairs with both DRB1_DQB1 match (RR of grade III-IV, 1.32, $P < .001$; and RR of grade II-IV, 1.34, $P < .001$). HLA-DRB1 mismatch alone or HLA-DQB1 mismatch alone showed no significant difference in either grade III-IV or grade II-IV acute GVHD from DRB1_DQB1 match, respectively. Thus, DRB1_DQB1 mismatch induced a greater effect on acute GVHD than would be expected from the independent effect of either HLA-DRB1 or HLA-DQB1 mismatch alone.

As with acute GVHD, stratified analysis of both HLA locus matching showed that pairs with DRB1_DQB1 mismatch were at significantly higher risk of mortality than pairs with DRB1_ DQB1 match (RR 1.17, $P < .001$) (Table 4). In contrast, risk with HLA-DRB1 mismatch alone or HLA-DQB1 mismatch alone was not significantly different from that with DRB1_DQB1 match (RR 1.04, $P = .662$ and RR 1.04, $P = .532$, respectively).

The risk of double HLA locus mismatch combinations other than DRB1_DQB1 for grade III to IV acute GVHD and mortality were analyzed. As shown in supplemental Table 3, none of these double mismatch combinations revealed an epistatic effect of double HLA locus mismatch.

Table 3. Effect of HLA locus matching on leukemia relapse, engraftment, and mortality

		Leukemia relapse†				Engraftment‡				Mortality			
HLA	Match or mismatch*	N	RR	95% CI	P	N	RR	95% CI	P	N	RR	95% CI	P
A	Match	4847	1.00		.381	6898	1.00		.035	7048	1.00		<.001
	Mismatch	606	0.92	0.76-1.11		851	0.93	0.87-0.99		850	1.29	1.17-1.42	
B	Match	5163	1.00		.493	7320	1.00		.146	7475	1.00		<.001
	Mismatch	290	0.91	0.69-1.20		429	0.93	0.84-1.03		423	1.27	1.11-1.45	
C	Match	3865	1.00		<.001	5511	1.00		.049	5565	1.00		<.001
	Mismatch	1588	0.70	0.61-0.80		2238	0.95	0.90-1.00		2333	1.21	1.13-1.30	
DRB1	Match	4045	1.00		.468	5763	1.00		.212	5878	1.00		.125
	Mismatch	1408	0.93	0.76-1.14		1986	0.95	0.89-1.03		2020	1.09	0.98-1.21	
DQB1	Match	3924	1.00		.974	5583	1.00		.014	5681	1.00		.145
	Mismatch	1529	1.00	0.83-1.22		2166	0.91	0.85-0.98		2217	1.08	0.97-1.19	
DPB1	Match	1792	1.00		<.001	2531	1.00		.126	2604	1.00		.349
	Mismatch	3661	0.69	0.61-0.77		5218	0.97	0.92-1.01		5294	1.03	0.96-1.11	

Multivariable competing risk regression analyses were conducted to evaluate the impact of leukemia relapse and neutrophil engraftment, and a Cox proportional regression model was conducted for mortality. RR of respective HLA locus mismatches at the allele level was compared with HLA match adjusted with other HLA locus matching and the clinical factors listed in Table 1 for leukemia relapse and mortality. Transplanted cell number and ABO blood type matching were added for neutrophil engraftment.

*GVH direction for leukemia relapse and mortality; HVG direction for engraftment.

†At 5 years after transplantation.

‡Neutrophil recovery to successive >500 per microliter measurement at 3 time points in 100 days.

The same results were obtained using the same stratified analysis of HLA-DRB1 and -DQB1 with serological HLA-A, -B, and -DR match pairs (supplemental Table 4).

Effect of HLA locus matching on leukemia relapse

The occurrence of leukemia relapse within 5 years after transplantation was analyzed in patients with AML, ALL, and CML. RR of HLA allele mismatch compared with HLA allele match for leukemia relapse was low with high significance in HLA-C (RR 0.70, $P < .001$) and -DPB1 (RR 0.69, $P < .001$), but was not significant in HLA-A, -B, -DRB1, or -DQB1 (Table 3).

Independence of GVL effect of HLA-DPB1 mismatch from chronic GVHD

As described in the previous paragraph, HLA-DPB1 mismatch induced the GVL effect, but did not induce chronic GVHD. Chronic GVHD also induced the GVL effect. Therefore, the GVL effect of HLA-DPB1 matching in relation to chronic GVHD was analyzed in 2129 leukemia patients with HLA-A, -B, -C, -DRB1, and -DQB1 allele complete match donors who survived 100 or more days after transplantation. Multivariate competing risk regression analysis, including HLA-DPB1 matching and chronic GVHD, were performed with chronic GVHD treated as a time-dependent covariate (Table 5). Both limited-type chronic GVHD and extensive-type chronic GVHD were associated with a significantly lower leukemia relapse risk than no chronic GVHD. Furthermore, 1 and 2 DPB1 allele mismatch was associated with a significantly lower leukemia relapse risk than HLA-DPB1 match. Interaction analysis between HLA-DPB1 matching and chronic GVHD was not significant (RR 1.26, 95% CI 0.85-1.88, $P = .255$), indicating the lack of any effect modification between HLA-DPB1 matching and chronic GVHD.

When acute GVHD was added to this analysis, RR of grade III-IV acute GVHD and grade II-IV acute GVHD was 0.77 (95% CI 0.57-1.04, $P = .091$) and 0.82 (95% CI 0.68-0.99, $P = .038$), respectively. Thus, the effect of acute GVHD on leukemia relapse was not significant in patients who survived more than 100 days after transplantation.

Effect of HLA locus matching on neutrophil engraftment

Engraftment risk of neutrophils at 100 days after transplantation was assessed in all patients. Although RR of engraftment by HLA locus mismatch in the HVG direction showed the relatively lower risk range of 0.91 to 0.97 compared with HLA locus match in all 6 HLA loci, there was no significant HLA locus matching for neutrophil engraftment (Table 4).

Effect of multiple HLA locus mismatch on acute GVHD and survival

As the above HLA locus matching analysis indicated that multiple HLA locus mismatch was associated with a higher risk of adverse

Table 4. Stratified analysis of HLA-DRB1 and HLA-DQB1 matching on acute GVHD and survival

		Acute GVHD (Grade III-IV)†			Acute GVHD (Grade II-IV)†			Mortality†		
HLA matching*	N	RR	95% CI	P	RR	95% CI	P	RR	95% CI	P
DRB1 match and DQB1 match	5356	1.00			1.00			1.00		
DRB1 mismatch and DQB1 match	325	0.98	0.74-1.28	.866	1.19	1.00-1.42	.046	1.04	0.88-1.22	.662
DRB1 match and DQB1 mismatch	522	0.92	0.73-1.16	.482	1.05	0.91-1.21	.517	1.04	0.92-1.19	.532
DRB1 mismatch and DQB1 mismatch	1695	1.32	1.16-1.50	<.001	1.34	1.23-1.46	<.001	1.17	1.08-1.27	<.001

Multivariable competing risk regression analyses were conducted to evaluate the impact of acute GVHD and Cox proportional regression model for mortality. RR of the combination of HLA-DRB1 and/or -DQB1 mismatch was compared with HLA-DRB1 and -DQB1 match. Adjusted confounders were HLA-A, -B, -C, and -DPB1 locus matching and the clinical factors listed in Table 1.

*GVH direction.

†Survived 7 or more days.

Table 5. Effect of chronic GVHD and HLA-DPB1 matching on leukemia relapse

	N	RR	95% CI	P
HLA-DPB1				
Match*	804	1.00		
1-allele mismatch*	971	0.70	0.58-0.84	<.001
2-allele mismatch*	354	0.54	0.41-0.72	<.001
Chronic GVHD				
No	1232	1.00		
Limited type	345	0.56	0.42-0.74	<.001
Extensive type	552	0.46	0.36-0.58	<.001

Multivariate competing risk regression analysis including HLA-DPB1 matching and chronic GVHD was performed by treating chronic GVHD as a time-dependent covariate adjusted for the clinical confounders listed in Table 1.

*GVH direction.

clinical outcomes of acute GVHD and survival, we next explored the appropriate HLA mismatch locus combination which revealed the effect of the number of HLA mismatch loci for acute GVHD and survival. The number of HLA 1-allele mismatches was summed after exclusion of 2-allele mismatches in each HLA locus. The combination of HLA-DRB1 1-allele mismatch and HLA-DQB1 1-allele mismatch (DRB1_DQB1 mismatch) was adopted and treated as 1 HLA locus mismatch.

The cumulative incidence curve of grade III-IV acute GVHD by the number of HLA-A, -B, -C, -DPB1 locus mismatches and DRB1_DQB1 mismatch showed a clear-cut risk difference which discriminated 0, 1, 2, 3, and 4 HLA locus mismatches (Figure 1A). Specifically, compared with 0 mismatches (n = 1476), RRs for grade III-IV acute GVHD were 1.37 with 1 mismatch (n = 2549), 2.19 with 2 mismatches (n = 1377), 2.82 with 3 mismatches (n = 415), and 3.25 with 4 mismatches (n = 60) ($P < .001$).

To clarify the risk of a 2 HLA loci single-mismatch combination, each 2 mismatch combination was compared with the combination of HLA-A and -C mismatch for grade III-IV GVHD. As shown in supplemental Table 5, the risk of double mismatch combination pairs showed no significant differences, except DRB1_DQB1 mismatch and -DPB1 mismatch combination, albeit that the number of some of these combinations was too small for any precise evaluation of risk.

The most clear-cut risk difference discriminating 0, 1, 2, 3, and 4 HLA locus mismatches is seen in the Kaplan-Meier curve for survival by the number of HLA locus mismatches of HLA-A, -B, -C, and DRB1_DQB1 (Figure 1B). Compared with 0 mismatches (n = 4076), the RR for mortality was 1.28 with 1 mismatch (n = 2352), 1.57 with 2 mismatches (n = 850), and 1.73 with 3 mismatches (n = 130) ($P < .001$). To clarify the risk of a 2 HLA loci single-mismatch combination, each 2 mismatch combination was compared with the combination of HLA-A and -C mismatch for mortality. As shown in supplemental Table 5, there were no significant differences between each double mismatch combination.

When HLA-DRB1 mismatch and HLA-DQB1 mismatch were added separately to this analysis, the survival curves of 1, 2, 3, 4, and 5 mismatches showed less clear-cut differences (Figure 1C).

Significant clinical factors other than HLA matching which affected transplant-related clinical outcomes

Significant variables ($P < .01$) other than HLA locus matching for acute GVHD, chronic GVHD, leukemia relapse, neutrophil engraftment, and mortality are listed in Table 6. Patient age affected acute GVHD, chronic GVHD and mortality, and donor age affected chronic GVHD and mortality. Compared with ALL, CML showed a lower risk of chronic GVHD, leukemia relapse and mortality, and a higher risk of neutrophil engraftment. AML showed a lower risk of mortality, and aplastic anemia showed a lower risk of acute GVHD, chronic GVHD and mortality. A reduced conditioning regimen

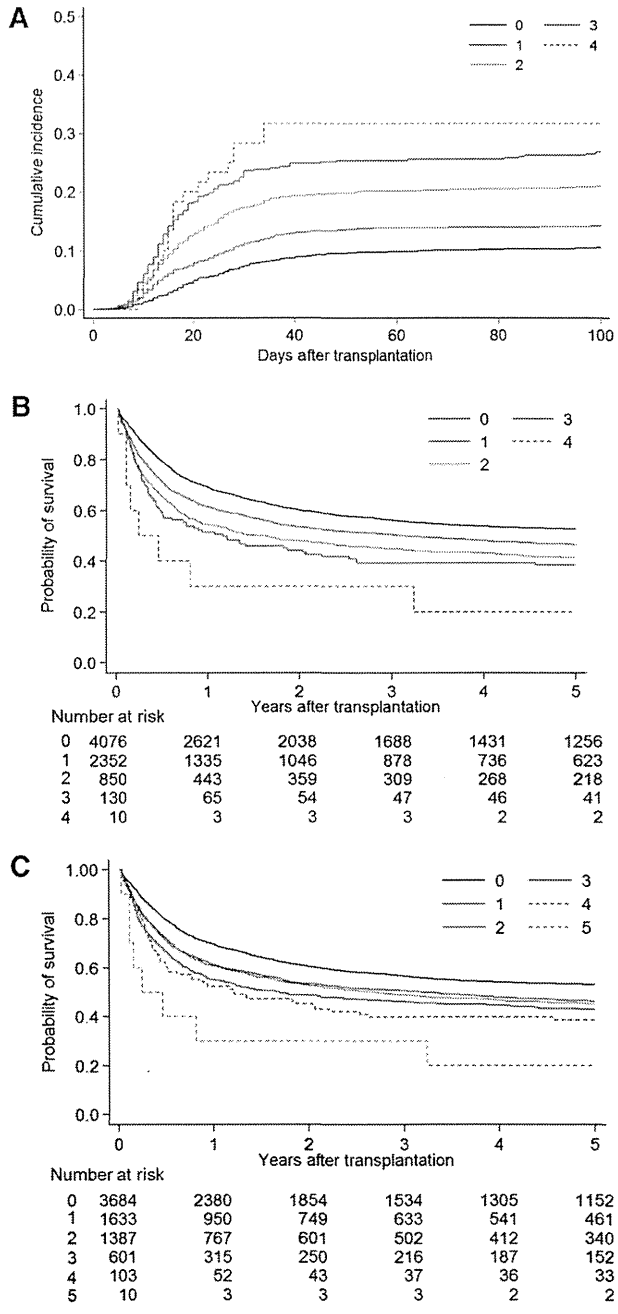

Figure 1. Acute GVHD and survival curve by the number of multiple HLA locus mismatches. The number of HLA 1-allele mismatches in the GVH direction, with exclusion of 2-allele mismatches, in each HLA locus was summed. (A) Cumulative incidence of grade III-IV acute GVHD by the mismatch number of HLA-A, -B, -C, -DRB1_DQB1, and -DPB1 at the allele level in the GVH direction. DRB1_DQB1: both HLA-DRB1 mismatch and HLA-DQB1 mismatch treated as 1 mismatch. 0: no mismatch (n = 1476); 1: 1 mismatch (n = 2549); 2: 2 mismatches (n = 1379); 3: 3 mismatches (n = 415); 4: 4 mismatches (n = 60). Cumulative incidence at 100 days was 0, 11% (95% CI, 9%-12%); 1, 14% (13%-16%); 2, 21% (19%-23%); 3, 27% (23%-31%); and 4, 32% (20%-44%). (B) Kaplan-Meier curve of survival by the mismatch number of HLA-A, -B, -C, and -DRB1_DQB1 at the allele level. Survival rate at 5 years was 0, 53% (95% CI, 51%-54%); 1, 46% (44%-49%); 2, 41% (38%-45%); 3, 38% (30%-47%); and 4, 20% (3%-47%). (C) Kaplan-Meier curve of survival by the mismatch number of HLA-A, -B, -C, -DRB1, and -DQB1 at the allele level.

Table 6. Significant factors other than HLA locus matching for clinical outcomes

Outcomes, Significant factor ($P < .01$)	N	RR	95% CI	P
Acute GVHD (grade III-IV)				
Patient age, year linear	7898	0.99	0.99-1.00	<.001
Disease				
ALL (Ref.)	1861	1.00		
Aplastic anemia	489	0.41	0.26-0.64	<.001
Conditioning				
Myeloablative (Ref.)	6653	1.00		
Reduced intensity	1245	1.26	1.07-1.50	.007
Sex matching				
Female to male (Ref.)	1494	1.00		
Female to female	1442	0.77	0.64-0.92	.005
Chronic GVHD				
Patient age, year linear	6528	1.01	1.00-1.01	<.001
Donor age, year linear	6528	1.00	1.00-1.00	<.001
Disease				
ALL (Ref.)	1568	1.00		
CML	813	1.28	1.13-1.46	<.001
Aplastic anemia	425	0.64	0.46-0.89	.008
Transplanted year				
1993-2000 (Ref.)	1865	1.00		
2006-2010	2117	0.74	0.65-0.83	<.001
Leukemia relapse				
Disease				
ALL (Ref.)	1861	1.00		
CML	983	0.49	0.39-0.60	<.001
Leukemia risk				
Standard (Ref.)	2508	1.00		
High	2772	2.62	2.31-2.98	<.001
Transplanted year				
1993-2000 (Ref.)	1815	1.00		
2001-2005	2079	1.34	1.14-1.56	<.001
2006-2010	1559	1.31	1.09-1.57	.004
Neutrophil engraftment				
Disease				
ALL (Ref.)	1831	1.00		
CML	959	0.90	0.84-0.97	.005
GVHD prophylaxis				
Cyclosporin based (Ref.)	2998	1.00		
Tacrolimus based	4716	1.12	1.07-1.18	<.001
Leukemia risk				
Standard (Ref.)	2486	1.00		
High	2703	0.81	0.77-0.85	<.001
Sex matching				
Female to male (Ref.)	1462	1.00		
Male to male	3182	1.10	1.03-1.16	.002
Male to female	1686	1.12	1.05-1.20	.001
ABO blood type matching				
Match (Ref.)	3455	1.00		
Major mismatch	1452	0.88	0.83-0.94	<.001
Transfused nuclear cell no./weight, kg, $\times 10^{E8}$				
<2.0 (Ref.)	1038	1.00		
2.0-4.0	4999	1.34	1.26-1.42	<.001
≤4.0	1068	1.42	1.31-1.55	<.001
Mortality				
Patient age, year linear	7898	1.02	1.02-1.02	<.001
Donor age, year linear	7898	1.01	1.01-1.02	<.001
Disease				
ALL (Ref.)	1861	1.00		
AML	2609	0.81	0.74-0.89	<.001
CML	983	0.72	0.63-0.81	<.001
MDS	841	0.50	0.40-0.64	<.001
Other leukemia	312	0.68	0.52-0.89	.005
Lymphoid malignancy	542	0.54	0.42-0.70	<.001
Aplastic anemia	489	0.30	0.23-0.40	<.001
Leukemia risk				
Standard (Ref.)	2508	1.00		
High	2772	2.19	2.01-2.39	<.001
Sex matching				
Female to male (Ref.)	1494	1.00		
Female to female	1442	0.81	0.72-0.90	<.001
Transplanted year				
1993-2000 (Ref.)	2311	1.00		
2001-2005	3084	0.81	0.74-0.89	<.001
2006-2010	2503	0.67	0.60-0.75	<.001

Multivariable competing risk regression analyses were conducted to evaluate the impact of acute GVHD, chronic GVHD, leukemia relapse and neutrophil engraftment, and a Cox proportional regression model for mortality. RR of respective factors was compared with the reference factor adjusted by HLA locus matching and clinical factors. Factors with significance ($P < .01$) were listed. RR of all variables is shown in supplemental Table 6.

Ref., reference factor.

showed a higher risk of acute GVHD (grade III-IV) compared with a myeloablative regimen. Tacrolimus-based GVHD prophylaxis showed a higher rate of neutrophil engraftment compared with cyclosporine-based GVHD prophylaxis, but no increase for acute GVHD and chronic GVHD. Sex matching conversely affected acute GVHD and neutrophil engraftment. ABO blood type matching and transplanted cell number affected neutrophil engraftment. The passage of time, reflecting an improvement in clinical selection for variables, was associated with a lower risk of mortality as a whole. RR of all variables for each factor are shown in supplemental Table 6.

Discussion

In this study, the accumulation of UR-HSCT clinical data and HLA retyping data through the JMDP allowed us to analyze biological immune responses of transplant-related events by HLA locus matching at the allele level. As data for some of the previously identified HLA alleles were no longer up to date, precise assessment of HLA matching required that we renew HLA allele types to meet the recent HLA nomenclature. We performed HLA allele typing for all HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1. In addition, to elucidate the biological immune responses, we strictly restricted pairs to non-T-cell–depleted bone marrow as stem cell source and to Japanese pairs as ethnic background.

Significant RRs of HLA allele mismatch compared with match were HLA-A, -B, -C and -DPB1 for grade III-IV acute GVHD; HLA-C for chronic GVHD; HLA-C and HLA-DPB1 for leukemia relapse; and HLA-A, -B, -C for mortality. Furthermore, stratified analysis of HLA-DRB1 and -DQB1 revealed that HLA-DRB1_DQB1 double mismatch was a significant RR for severe acute GVHD and mortality. These findings supersede previous JMDP studies[2,4,5] and provide a rationale for the development of an algorithm for unrelated donor selection.

HLA-A and/or -B locus mismatch induced significant severe acute GVHD but not the GVL effect, and resulted in a lower survival rate than in HLA match pairs. Since the first report from the JMDP showing the risk of HLA-A and/or -B for acute GVHD and survival, both the selection of HLA-A and/or -B mismatch donors and the impact of

this mismatch have dramatically decreased. In spite of this information bias, HLA-A and/or -B allele mismatch should be considered in donor selection and GVHD prophylaxis as a high-risk HLA locus of severe acute GVHD and mortality. The NMDP[6,7] and IHWG reports[10] also indicated the risk of HLA-A and/or -B mismatch.

HLA-C mismatch induces not only a high risk of acute GVHD but also a high risk of chronic GVHD and low risk of leukemia relapse. When an HLA-C mismatch donor is considered for the induction of GVL effect in general practice, the risk of acute GVHD and chronic GVHD should be kept in mind. This effect of HLA-C mismatch on leukemia relapse and survival confirms findings of previous JMDP[5,25] and NMDP reports.[6] In addition to T-cell recognition of the mismatched amino acid difference in HLA-C molecules,[14] NK-cell receptor KIR2DL ligand mismatch should also be considered, as described elsewhere.[5,26] The effect of KIR ligand mismatch remains controversial worldwide. Further analysis of HLA-C allele mismatch combination in conjunction with KIR receptor using JMDP pairs and comparison with non-JMDP pairs will help to elucidate the mechanism of HLA-C and KIR-related immunologic reaction and solve these discrepancies.

Our stratified analysis showed that the concurrent presence of HLA-DRB1 mismatch and HLA-DQB1 mismatch was associated with a high risk of severe acute GVHD and mortality, whereas the presence of HLA-DRB1 mismatch or HLA-DQB1 mismatch only did not induce a significantly higher risk of severe acute GVHD or survival. This epistasis of 2 HLA loci mismatch needs to be interpreted with care. In particular, the relatively small number of DRB1 alone mismatch pairs (n = 325) might have limited the statistical power. An additional consideration is that no other HLA 2 locus mismatch combination showed such an epistatic effect of DRB1 and DQB1 on the risk of severe acute GVHD and mortality (supplemental Table 3). Interaction of the HLA-DQB1 molecule with that of HLA-DR groups might evoke unique immune reactions related to allogeneic transplantation for severe acute GVHD. As reported by Fernández-Viña et al,[27] the effect of the low expression of HLA loci, not only of DP, DQ but also the DRB3/4/5 locus, needs to be explored.

As also reported by Shaw et al,[8] the present study found that HLA-DPB1 mismatch induced acute GVHD and the GVL effect, but did not affect survival. HLA-DP antigen was originally typed using the in vitro–primed lymphocyte test. From this, HLA-DPB1 and its matching are known to play a distinct biological role in immunologic reactions. Indeed, the GVL effect in HLA-DPB1 mismatch combination in our previous analysis provided a rationale to explain the induction of the GVL effect and less acute GVHD.[25] In addition, our present results show for the first time that HLA-DPB1 mismatch and the occurrence of chronic GVHD affect the GVL effect independently of each other. The mechanism of the GVL effect induced by T-cell recognition of the HLA-DPB1 allele mismatch might differ from that induced by chronic GVHD. Potential candidates for the molecular implications of acute GVHD and the GVL effect include the high-risk HLA-DPB1 mismatch combinations for severe acute GVHD reported from the JMDP[14,25] and the effect of T-cell-epitope matching at HLA-DPB1 reported by Fleischhauer et al.[16]

When the impacts of the respective HLA locus matching described above are taken together, RR of mismatch of HLA class I loci is heightened, with a range of RR 1.29 to 1.63 for severe acute GVHD and RR 1.21 to 1.27 for mortality. For HLA class II loci, mismatch of double HLA-DRB1 and -DQB1 should be considered, with RR 1.32 for severe acute GVHD and 1.14 for mortality. Thus, appropriate combinations of HLA loci need to be selected according to the risk of each HLA locus and the interaction of HLA-DRB1 and -DQB1 for donor selection.

The number of multiple mismatches of HLA-A, -B, -C, -DRB1_DQB1 and -DPB1 showed good predictive value for the risk of severe acute GVHD. Furthermore, prediction of the risk of mortality after transplantation should consider the number of multiple mismatches of HLA-A, -B, -C, and -DRB1_DQB1 locus, and not of HLA-A, -B, -C, -DRB1, and -DQB1. This mismatch score is in agreement with reports from the NMDP[6,7,11] and Loiseau et al[28] showing that mismatch of HLA-DQB1 demonstrated an additive adverse effect in outcomes. Our analysis using the present data set is consistent with findings from a recent report[29] which showed a significant risk with single HLA-DRB1 mismatch using the Japanese HSCT dataset in leukemia patients with HLA-A, -B, -C and -DRB1 allele data.

Our analysis also provides further information for personalized unrelated donor selection. In cases where the transplant team is particularly concerned about the prevention of severe acute GVHD, leukemia relapse or early mortality, the specific HLA locus mismatches and number of mismatched locus should be considered with regard to the patient's disease, disease status, and clinical condition. The benefit of HLA-C mismatch and HLA-DPB1 mismatch for a specific GVL effect in leukemia patients is noted.

A number of other important factors will also impact clinical outcomes and change the magnitude of the HLA barrier. In the present study, clinical risk factors other than HLA matching are shown in Table 6. The magnitude of risks for HLA locus mismatch is compatible with that for clinical factors as a whole.

Candidates range widely, from ethnicity of the donor and patient[30] to HLA haplotype[12,13] and other genetic polymorphisms both inside and outside the HLA region.[31-33] Clinical risk factors in the present study agree with those reported previously, including procedures for GVHD prophylaxis, intensity of the conditioning regimen,[34] disease,[35,36] leukemia relapse risk, and stem cell source.[37] It will be interesting to determine whether these candidates shift the HLA barrier quantitatively and maintain the same divergent effect of each HLA locus, or qualitatively alter the HLA locus-specific barrier. As unrelated peripheral blood stem cell transplantation was not facilitated by the JMDP during the period of this study, we were unable to analyze the data for unrelated PBSCT. PBSCT might heighten the threshold of the HLA barrier, as reported by the NMDP.[37] Analysis for unrelated cord blood transplantation compared with unrelated donor transplantation[38,39] might shed light on the latter possibility and help elucidate the altered immune mechanisms which cause transplant-related events.

Our homogeneous cohort was restricted to Japanese pairs, which allowed us to elucidate biological responses based on this particular genetic background. However, individual ethnic groups present distinct HLA allele and HLA haplotypes, and these differences in the ethnic background of patient and donor might impact transplant-related clinical outcomes.[40] Our findings need to be validated using unrelated donor transplantation data for other ethnic groups.

In conclusion, we clearly determined the HLA locus mismatches responsible for diverse transplant-related immunologic events. Furthermore, we provide a rationale for the development of an algorithm for unrelated donor selection.

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Authorship

Contribution: Y.M., K. Kashiwase, K. Matsuo, M.M., T.I., H. Saji, S.K., Y.K., and T.S. participated in the design of the study; K. Kashiwase, F.A., and T.Y. performed the histocompatibility analysis; M.O., N.D., T.E., Y.M., K. Miyamura, T.M., H. Sao, Y.A., and K. Kawa organized and collected the clinical data and samples for transplantation; Y.M., S.M., and K. Matsuo performed statistical data analysis; Y.M., S.M., and K. Kashiwase performed the analysis and wrote the paper; and all authors checked the final version of the paper.

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Biological significance of HLA locus matching in unrelated donor bone marrow transplantation

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Donor Lymphocyte Infusion for the Treatment of Relapsed Acute Myeloid Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Analysis by the Adult Acute Myeloid Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation

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A B S T R A C T

Because the efficacy of donor lymphocyte infusion (DLI) for acute myeloid leukemia (AML) relapse after allogeneic hematopoietic stem cell transplantation (HSCT) remains uncertain, especially in the Asian population, a nationwide registry study was retrospectively performed by the Adult AML Working Group of the Japan Society for Hematopoietic Cell Transplantation to identify the factors affecting the patient survival after DLI. Among 143 adult AML patients who received DLI for the treatment of first hematological relapse after HSCT, the overall survival rates at 1 year, 2 years, and 5 years were 32% ± 4%, 17% ± 3%, and 7% ± 3%, respectively. Complete remission (CR) at the time of DLI, which was obtained in 8% of the patients, was the strongest predictive factor for survival after DLI. Therefore, long-term survival after DLI was achieved almost exclusively in patients who successfully achieved a CR before DLI, indicating the limited efficacy of DLI in a minority of patients.

INTRODUCTION

Relapse remains a major obstacle to the survival of patients with acute myelogenous leukemia (AML) after allogeneic hematopoietic stem cell transplantation (HSCT), accounting for 20% to 50% of the primary causes of death [1,2]. Although the best way to manage AML relapse after allogeneic HSCT is unclear, donor lymphocyte infusion (DLI) is 1 of the most common interventions used for AML relapse, with the expectation of inducing a graft-versus-leukemia (GVL) effect [2-4]. However, treatment success for AML relapse is limited, with overall survival (OS) rates of 10% to 20% at 3 years in previous studies [2-8]. To predict the efficacy of DLI in advance may lead to the selection of different treatments, including second HSCT, for patients predicted to be unresponsive to DLI. Until now, large-scale studies to analyze the risk factors for the success of DLI have been scarce, especially in the Asian population. The aim of this

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study was to retrospectively identify the factors affecting the efficacy of DLI for adult patients with a first hematological relapse after allogeneic HSCT, using national registry-based data of the Transplant Registry Unified Management Program (TRUMP) in Japan.

PATIENTS AND METHODS

Data Collection

The data for 14,286 Japanese patients with AML who underwent HSCT were obtained from the TRUMP in Japan [9]. Data regarding white blood cell count at diagnosis, blast count and chimerism at relapse, and cell dose of DLI were not available for this cohort. Inclusion was based on the following criteria: first allogeneic, bone marrow (BM) or peripheral blood stem cell (PBSC) HSCT between 1991 and 2011, age ≥ 16 years at transplantation, and DLI recipients after the first hematological relapse after HSCT without precedence of a second transplantation. Patients with myelodysplastic syndrome, secondary AML from myelodysplastic syndrome, or a subsequent relapse of AML were excluded. Patients never in remission at transplantation were excluded. A total of 143 patients met the criteria for study inclusion. The study design was approved by the TRUMP data management committee of the Japan Society for Hematopoietic Cell Transplantation and the institutional review board of Kanazawa University Hospital, where this study was organized.

Definitions

DLI was defined as transfusion of unstimulated lymphocyte concentrates, collected from the original stem cell donor as buffy coat preparations. According to a previous study [3], the transfusion of unmanipulated mobilized PBSC concentrates was also defined as DLI, if no prophylactic immunosuppressive medication was given, whereas the infusion of donor PBSC or BM after conditioning the patient with prophylactic immunosuppression for graft-versus-host disease (GVHD) prevention was defined as a second HSCT. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to traditional criteria [10,11]. *Complete remission* (CR) was defined by normal values for the absolute neutrophil count (>1000/μL) and platelet count (>100,000/μL), independence from red cell transfusion, and absence of signs of leukemia without ongoing antileukemic therapy, based on the revised recommendations of the international working group [12]. The classification of conditioning regimens as to whether they were myeloablative or reduced-intensity was based on the report by the Center for International Blood and Marrow Transplant Research [13]. Cytogenetic subgroups were classified according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria [14].

Endpoints

The primary study endpoint was to identify the factors affecting the OS after DLI.

Statistical Analysis

All statistical analyses were performed with the EZR software package (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [15]. Variables included the recipient's age at time of transplantation, sex, pretransplantation cytomegalovirus serostatus, disease characteristics (French–American–British classification [FAB] and cytogenetics), donor characteristics (age, sex, ABO and HLA compatibility), transplantation characteristics (year of transplantation, disease status at transplantation, conditioning, source of stem cells, acute GVHD, and/or chronic GVHD before DLI), and relapse and DLI characteristics (interval from transplantation to relapse, interval from relapse to DLI, chemotherapy before DLI, disease status at DLI, and acute GVHD after DLI). The median was used as the cutoff point for continuous variables. The chi-square test and the Mann-Whitney U test were used to compare data between 2 groups. The probability of OS was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of acute and chronic GVHD were analyzed using a cumulative incidence analysis [16], while considering death without acute GVHD and death without chronic GVHD as respective competing risks. All factors found to be significant in the univariate analyses ($P \leq .10$) were included in multivariate Cox hazard models. For both the univariate and multivariate analyses, P values were 2-sided and the outcomes were considered to be significant for values of $P \leq .05$.

RESULTS

Patient Characteristics

A total 143 patients with AML who received DLI for treatment of a first hematological relapse after allogeneic HSCT were included in the study (Table 1). The median time interval from HSCT to relapse was 149 days (range, 28 to 2153) and from relapse to DLI was 37 days (range, 0 to 841). Only 8% of patients had obtained CR at the time of DLI. One single infusion of DLI was given to 76% of patients, and the remaining patients received 2 or more infusions.

Table 1
Characteristics of the Adult Patients who received DLI for the Treatment of Their First Hematological Relapse after HSCT for AML

No. of patients	143
Age at relapse, median (range), yr	49 (16-67)
Cytogenetics	
Good	20 (14)
Intermediate	81 (57)
Poor	42 (29)
Follow-up of survivors after DLI, median (range), d	459 (73-4377)
Interval from relapse to DLI, median (range), d	37 (0-841)
Extramedullary relapse	
No	131 (92)
Yes	12 (8)
Acute GVHD present at relapse	
No	69 (48)
Yes	71 (50)
Data missing	3 (2)
Chronic GVHD present at relapse	
No	95 (66)
Yes	28 (20)
Not evaluated or missing	20 (14)
Acute or chronic GVHD present at relapse	
No	62 (43)
Yes	79 (55)
Data missing	2 (1)
Chemotherapy before DLI	
No	21 (14)
Yes	55 (38)
Data missing	67 (47)
Status at DLI	
Active disease or aplasia	132 (92)
Complete remission	11 (8)
Transfusions, n	
1	109 (76)
2	22 (15)
≥ 3	12 (8)
Acute GVHD after DLI	
Yes	26 (18)
No	117 (82)
Cause of death	
Infection	11 (9)
Interstitial pneumonia	4 (3)
GVHD	2 (2)
Hemorrhage	6 (5)
Organ failure	11 (9)
Persistent or relapsed leukemia	86 (71)
Data missing	1 (1)

Data presented are n (%), unless otherwise indicated.

OS after DLI

In the 143 relapse patients who received DLI, the 1-year, 2-year, and 5-year OS rates from DLI were 32% ± 4%, 17% ± 3%, and 7% ± 3%, respectively. Among the 143 patients, 121 patients (85%) died after DLI, and the main cause of death was persistent or relapsed leukemia in 86 patients (71%), infections in 11 (9%), organ failure in 11 (9%), hemorrhage in 6 (5%), interstitial pneumonia in 4 (3%), and GVHD in 2 patients (2%). The median follow-up of the remaining 22 survivors after DLI was 459 days (range, 73 to 4377).

The factors significantly associated with a shorter OS after DLI based on the univariate analysis included male sex, sex match of the donor and recipient in contrast to a male donor for a female recipient, HLA mismatch of the donor and recipient, a related PBSC recipient at HSCT compared with a

Table 2
Results of Univariate Analysis of the Risk Factors for Survival after DLI

Characteristic	OS at One Year		OS at Two Years		*P* Value
	%	SE	%	SE	
Overall	32%	4%	17%	3%	
Patient age, yr					
<49	24%	5%	17%	5%	
≥49	21%	5%	9%	4%	.25
Patient sex					
Female	29%	6%	15%	5%	
Male	18%	4%	12%	4%	.02
Donor age, yr					
<37	25%	6%	15%	5%	
≥37	19%	5%	12%	5%	.47
Donor sex					
Male	24%	5%	14%	4%	
Female	20%	6%	12%	5%	.40
Sex matching					
Male donor to female recipient	35%	8%	21%	7%	
Female donor to male recipient	22%	8%	18%	8%	.07
Matched	16%	4%	9%	3%	.02
ABO matching					
Matched	22%	5%	13%	4%	
Major mismatched	36%	13%	NA	NA	.16
Minor mismatched	13%	7%	9%	6%	.76
Major-minor mismatched	22%	14%	NA	NA	.77
ABO major mismatching					
No	20%	4%	12%	3%	
Yes	30%	10%	NA	NA	.16
ABO minor mismatching					
No	24%	4%	16%	4%	
Yes	16%	6%	8%	5%	.71
HLA matching					
Matched	25%	4%	16%	4%	
Mismatched	16%	7%	NA	NA	.05
Type of HLA-matched donor					
Related	23%	5%	13%	4%	
Unrelated	29%	8%	25%	8%	.88
Source of stem cells					
Related BM	30%	8%	15%	6%	
Related PBSC	17%	5%	10%	4%	.03
Unrelated BM	24%	7%	21%	7%	.38
Status at transplantation					
CR1 or CR2	25%	5%	13%	4%	
Advanced	21%	5%	15%	5%	.69
Pretransplantation CMV serostatus					
CMV positive recipient	26%	4%	15%	4%	
CMV negative recipient	10%	7%	0%	NA	.30
Year of transplantation					
<2006	23%	6%	16%	5%	
≥2006	22%	5%	NA	NA	.47
Cytogenetic subgroup					
Good	33%	11%	27%	10%	
Intermediate	26%	5%	14%	4%	.36
Poor	10%	6%	NA	NA	.04
Conditioning for transplantation					
Myeloablative	22%	5%	16%	4%	
Reduced intensity	23%	6%	10%	5%	.78
Interval from transplantation to relapse, mo					
<5	15%	4%	7%	3%	
≥5	34%	7%	23%	6%	.001
Acute GVHD at time of relapse					
No	22%	5%	13%	4%	
Yes	23%	5%	15%	5%	.74
Chronic GVHD at time of relapse					
No	19%	4%	12%	4%	
Yes	33%	10%	19%	8%	.34
Acute or chronic GVHD at time of relapse					
No	22%	5%	12%	4%	
Yes	24%	5%	15%	4%	.68
Extramedullary relapse					
No	28%	7%	18%	6%	
Yes	17%	14%	NA	NA	.99
Interval from relapse to DLI, d					
≥37	32%	6%	19%	5%	
<37	12%	4%	9%	4%	.003
Chemotherapy before DLI					
No	29%	11%	NA	NA	
Yes	26%	7%	21%	6%	.41
Status at DLI					
CR	100%	NA	100%	NA	
Active disease or aplasia	17%	3%	8%	3%	.00001
Acute GVHD after DLI					
No	32%	8%	23%	7%	
Yes	26%	9%	NA	NA	.89
Second transplantation after relapse					
No	22%	4%	15%	4%	
Yes	25%	9%	8%	6%	.80

NA indicates not available; CMV, cytomegalovirus.

related BM recipient, poor cytogenetics compared with good cytogenetics, a shorter interval (<5 months) from HSCT to relapse, a shorter interval (<37 days) from relapse to DLI, active disease or aplasia at the time of DLI, and a single infusion of DLI (Table 2). Other factors, such as the patient and donor age, presence of GVHD at relapse, and the development of acute GVHD after DLI, did not significantly influence OS after DLI.

A total of 26 patients developed acute GVHD after DLI (Table 1), with grade I GVHD in 15 patients, grade II in 5, grade III in 5, and grade IV in 1 patient. Of the 26 patients, 17 (69%), 3 (12%), 2 (8%), and 4 (15%) patients experienced acute GVHD after 1, 2, 3, and 4 courses of DLI, respectively. Eight (31%) of the 26 patients achieved disease-free survival after DLI, with durations ranging from 82 to 2258 days (median, 362 days), whereas 14 (12%) of the 121 patients without acute GVHD experienced disease-free survival. It may be noted that 5 (33%) of the 15 patients who developed grade I acute GVHD after DLI survived without disease over 2 years, and that 2 of the 26 patients who developed GVHD subsequently developed chronic GVHD, and both patients survived long-term without disease. Three other patients developed chronic GVHD without experiencing acute GVHD after DLI, and 2 of these 3 patients survived without disease for over 2 years. These data might suggest the association of GVHD after DLI with a substantial GVL effect.

The impact of GVHD on OS after DLI was evaluated as a time-dependent variable. In a multivariate analysis, a shorter interval from HSCT to relapse (hazard ratio, 1.76; 95% confidence interval, 1.10 to 2.57; $P = .02$) and active disease or aplasia at time of DLI (hazard ratio, 9.98; 95% confidence interval, 2.27 to 43.9; $P = .002$) remained significantly associated with a shorter OS (Table 3). The number of DLI infusions was closely linked to the interval from relapse to DLI and was, therefore, eliminated from the multivariate model. Disease stage at DLI had a relatively greater impact on OS after DLI compared with the interval from HSCT to relapse. In addition, among the 11 patients who had obtained CR at the time of DLI, 10 patients showed a longer interval from HSCT to relapse.

Accordingly, 3 prognostic groups were categorized as follows: CR at DLI, regardless of the interval from HSCT to

Table 3
Results of Multivariate Analysis of Risk Factors for Survival after DLI

Prognostic Factor	*P* Value	Hazard Risk for OS	95% CI
Female versus male	.49	1.24	.68-2.25
Male donor to female recipient versus female donor to male recipient	.69	1.20	.50-2.87
Male donor to female recipient versus sex matched	.36	1.35	.71-2.57
HLA matched versus HLA mismatched	.19	1.39	.85-2.27
Good cytogenetics versus intermediate cytogenetics	.21	1.45	.81-2.59
Good cytogenetics versus poor cytogenetics	.09	1.76	.92-3.39
Interval from transplantation to relapse, ≥ 5 mo versus <5 mo	**.02**	**1.68**	**1.10-2.57**
Interval from relapse to DLI, ≥ 37 d versus <37 d	.35	1.23	.80-1.90
Disease stage at DLI (complete remission versus active disease or aplasia)	**.002**	**9.98**	**2.27-43.9**

The bold results show values with a $P \leq .05$.
CI indicates the confidence interval.

relapse (group 1; n = 11), a longer interval (≥5 months) from HSCT to relapse but not in CR at DLI (group 2; n = 51), and others (group 3; n = 81) (Table 4, Figure 1). Among the patients who received DLI while in CR (group 1), the 2-year OS was as high as 100%, which was significantly better than that observed in those with a longer interval from HSCT to relapse without CR at DLI (group 2; 12%, $P < .001$) and a shorter interval from HSCT to relapse without CR at DLI (group 3; 4%, $P < .001$). Of note, no significant differences in OS after DLI were noted between group 2 and group 3 ($P = .13$). Accordingly, CR at the time of DLI was the strongest factor with a significant impact on OS after DLI.

DISCUSSION

Despite advances in decreasing the nonrelapse mortality (NRM) after allogeneic HSCT [17], there has been little progress in reducing the incidence of relapse or in improving the subsequent outcome. The long-term survival rate after relapse for patients who underwent transplantation with AML was reported to be 5% [18,19], although salvage therapy, such as withdrawal of immunosuppression, chemotherapy, radiotherapy, a second HSCT, and DLI have been attempted. However, durable remission occasionally develops after DLI for AML relapse [2-8]. The current nationwide study confirmed that AML patients who successfully achieved CR after relapse may benefit from DLI. Although the 5-year OS from relapse was low at 7%, a subset of patients who achieved CR before DLI had a significantly better 5-year OS of 50%, supporting the use of this treatment strategy [2,3] when a CR is obtained by salvage treatment, such as withdrawal of immunosuppression and/or salvage chemotherapy, and immediate consolidation with DLI should be recommended to improve the chance for long-term survival after AML relapse.

Previous studies have identified several factors that are associated with a good prognosis after DLI, including achievement of hematological remission before DLI, a lower tumor burden at relapse, female sex, favorable cytogenetics, remission at the time of DLI, a longer duration of remission after HSCT, and the absence of acute GVHD after HSCT [2,3,5,20-22], the most important of which were the tumor burden at relapse and the duration of remission after HSCT. The present study supports the importance of disease control before DLI.

One drawback is that the study was a retrospective registry analysis, limiting the risk factors that were available for analysis, including not only the blast count at relapse, but also the dose of mononuclear cells in the DLI grafts and the use of granulocyte colony–stimulating factor before harvesting the infused lymphocytes.

A second HSCT with or without DLI represents a good alternative treatment [3] because, at the current moment, the approaches expected to offer long-term survival for patients with AML who relapse after HSCT are confined to DLI and second HSCT. However, a second HSCT after myeloablative conditioning has historically been associated with poor survival, with higher NRM rates ranging from 25% to 45%. Recent approaches with a second HSCT after a reduced-intensity conditioning regimen minimized NRM rates to 0 to 30%, but this could be offset by the higher relapse rates after the second HSCT [1,23]. There have been few reports on whether DLI was superior to second HSCT, and a comparison of the efficacy of DLI and second HSCT for AML relapse is outside the scope of the present study. However, as shown Table 2, a second HSCT after DLI did not have a significant impact on the OS in patients with AML relapse.

Various modifications of DLI have been investigated, such as ex vivo activated DLI and earlier introduction of DLI [24-26]. A recent report [26] showed that preemptive DLI given when minimal residual disease (MRD) was detected effectively reverted MRD back to remission in all 16 treated patients with acute leukemia and offered long-term survival in 15 of the 16 patients without increasing the risk of GVHD development. Thus, early detection of potential disease progression by detecting MRD and subsequently performing DLI before overt relapse might be a better way to improve the success of HSCT for AML.

The major risk of DLI is the development of GVHD, which occurs in 40% to 80% of patients [20,27,28], placing patients at risk of significant morbidity and mortality. In the present study, the cumulative incidence of acute GVHD after DLI was as high as 82%. However, the development of acute GVHD after DLI did not significantly affect the long-term survival and it only caused 2% of the deaths. The majority of deaths resulted from original disease, which accounted for 79% of the deaths.

Table 4
Survival of Adult Patients Receiving DLI for Treatment of First Hematological Relapse after HSCT for AML (n = 143) Stratified according to Prognostic Group

Prognostic Group	n	OS at One Year		OS at Two Years		OS at Five Years		*P* Value
		%	SE	%	SE	%	SE	
Group 1: CR at DLI	11	100%	0%	100%	0%	50%	25%	
Group 2: Interval from transplantation to relapse, ≥5 mo, but not in CR at DLI	51	24%	6%	12%	4%	9%	5%	<.001
Group 3: Others	81	14%	4%	6%	3%	0%	0%	<.001

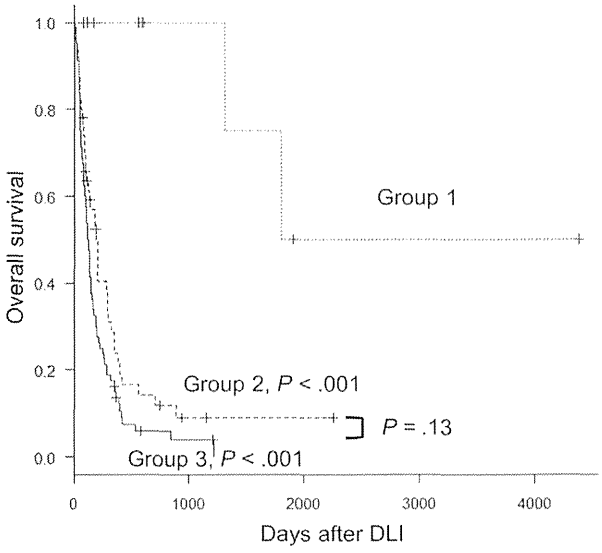

Figure 1. Survival after DLI according to the prognostic groups. Group 1 had a CR at DLI, regardless of the interval from HSCT to relapse (n = 11). Group 2 had a longer interval (≥5 months) from HSCT to relapse, but was not in CR at DLI (n = 51). Group 3 included the other patients (n = 81).

Despite the fact that there has been insufficient data about cases after DLI in the Asian population, several large-scale studies [2,3,6,7,27,29] that evaluated the efficacy of DLI for AML relapse after HSCT in non-Asian population have been reported. The OS rates from DLI in those studies ranged from 21% to 37%, 14% to 25%, 12% to 20%, and 10% to 15% at 1, 2, 3, and 5 years, respectively; comparable with the OS rates in the present study for the Asian population, which were 32%, 17%, 10%, and 7% at 1, 2, 3, and 5 years. The European Group for Blood and Marrow Transplantation Group [3] reported several factors that were associated with better OS, including remission at the time of DLI, as seen in the present study, bone marrow blasts less than 35% at relapse, female sex, and favorable cytogenetics. Therefore, there does not appear to be any major differences between the Asian and non-Asian populations in the context of the potent antileukemic effect of DLI for AML.

The nature of a retrospective, registry-based analysis implicates several limitations. There were missing data on the type of chemotherapy administered before DLI, no information about the cell doses and whether the DLI was a fresh infusion. Unfortunately, the present registry-based data do not include this information, and to collect such missing data is out of the scope of the present study. Therefore, further studies are warranted.

The present cohort does not include patients who received prophylactic immunosuppression, either after DLI or unmanipulated PBSC infusion, according to a previous report [3], to allow us to evaluate the pure GVL effect.

The results of this large retrospective study demonstrate that the efficacy of DLI is limited for the treatment of AML relapse after HSCT, and disease control at the time of DLI is critical for treatment success irrespective of operative chemotherapy before obtaining remission. However, the number of patients with CR was quite small (n = 11), and, therefore, conclusions should be considered with caution. New strategies to enhance and maintain the GVL effect of DLI while minimizing GVHD, which includes preemptive/prophylactic DLI before overt relapse, costimulation with cytokines or dendritic cells, and use of the leukemia-specific antibodies, such as gemtuzumab ozogamicin, should be considered.

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