

The New England Journal of Medicine

© Copyright, 1998, by the Massachusetts Medical Society

VOLUME 339

OCTOBER 22, 1998

NUMBER 17



EFFECT OF MATCHING OF CLASS I HLA ALLELES ON CLINICAL OUTCOME AFTER TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS FROM AN UNRELATED DONOR

TAKEHIKO SASAZUKI, M.D., PH.D., TAKEO JUJI, M.D., PH.D., YASUO MORISHIMA, M.D., PH.D., NAOKO KINUKAWA, M.S.,
HIDEHIKO KASHIWABARA, M.D., PH.D., HIDETOSHI INOKO, PH.D., TAKATO YOSHIDA, M.D., PH.D.,
AKINORI KIMURA, M.D., PH.D., TATSUYA AKAZA, M.S., NOBUHIRO KAMIKAWAJI, M.D., PH.D.,
YOSHIHISA KODERA, M.D., PH.D., AND FUMIMARO TAKAKU, M.D., PH.D., FOR THE JAPAN MARROW DONOR PROGRAM*

ABSTRACT

Background The requirements with respect to HLA compatibility and the relative importance of matching for individual class I and class II HLA alleles in the transplantation of hematopoietic stem cells from unrelated donors have not yet been established.

Methods We performed retrospective DNA typing of alleles at 11 polymorphic loci of HLA genes in 440 recipients of hematopoietic stem cells from unrelated donors who were serologically identical with their respective recipients for HLA-A, B, and DR antigens. Of these recipients, 80 percent had leukemia; the rest had lymphoma, marrow failure, or a congenital disorder.

Results Multivariate analysis showed that incompatibility for HLA-A alleles and incompatibility for HLA-C alleles were independent risk factors for severe acute graft-versus-host disease (GVHD) (HLA-A, $P=0.006$; HLA-C, $P=0.001$). Mismatching of HLA-A, but not of HLA-C, alleles was an independent risk factor for death ($P<0.001$). Mismatching of HLA-C alleles was a significant risk factor for relapse of leukemia ($P=0.035$). HLA-B disparity was a significant risk factor for both GVHD and death in the univariate analysis, but not in the multivariate analysis. Disparities in class II HLA alleles of the DRB1, DQA1, DQB1, DPA1, and DPB1 loci were not identified as significant risk factors for acute GVHD or death in the multivariate analysis.

Conclusions Genomic typing of class I HLA alleles adds substantially to the success of transplantation of hematopoietic stem cells from unrelated donors, even if the donors are serologically identical to their recipients with respect to HLA-A, B, and DR antigens. (N Engl J Med 1998;339:1177-85.)

©1998, Massachusetts Medical Society.

PATIENTS with malignant hematologic diseases, various forms of marrow failure, and certain congenital disorders can be successfully treated with transplantation of hematopoietic stem cells from HLA-identical siblings.¹⁻⁷ However, only 30 percent of patients for whom such treatment is the first choice have an HLA-matched donor within their immediate families. In studies in the United States of transplantation of marrow from unrelated donors, a single mismatch for HLA-A or B (class I HLA antigens) or HLA-DR (a class II HLA

From the Department of Genetics, Medical Institute of Bioregulation (T.S., N. Kamikawaji), and the Department of Medical Informatics, Faculty of Medicine (N. Kinukawa), Kyushu University, Fukuoka; the Japanese Red Cross Central Blood Center, Tokyo (T.J., T.A.); the Department of Hematology and Chemotherapy, Aichi Cancer Center Hospital, Nagoya (Y.M.); the Department of Surgery, Sakura National Hospital, Chiba (H.K.); the Department of Genetic Information, Division of Molecular Life Science, Tokai University School of Medicine, Kanagawa (H.I.); the Department of Microbiology and Immunology, Showa University School of Medicine, Tokyo (T.Y.); the Department of Tissue Physiology, Division of Adult Diseases, Medical Research Institute, Tokyo Medical and Dental University, Tokyo (A.K.); the Department of Internal Medicine, Japanese Red Cross Nagoya First Hospital, Nagoya (Y.K.); and Jichi Medical School, Tochigi (F.T.) — all in Japan. Address reprint requests to Dr. Sasazuki at the Department of Genetics, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan.

Other authors were Yoshiaki Nose, M.D., Ph.D. (Department of Medical Informatics, Faculty of Medicine, Kyushu University), Takashi Ono, M.D. (Department of Genetics, Medical Institute of Bioregulation, Kyushu University), Takeo Sakamaki, M.D., Ph.D. (Division of Clinical Research, Sakura National Hospital), Shunichi Kato, M.D., Ph.D. (Department of Pediatrics, Tokai University School of Medicine), Yuichi Akiyama, M.D. (Department of Pediatrics, Kyoto University School of Medicine, Kyoto), Shinichiro Okamoto, M.D., Ph.D. (Department of Medicine, Keio University School of Medicine, Tokyo), Hiroo Dohy, M.D., Ph.D. (Department of Medicine, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima), Mine Harada, M.D., Ph.D. (Second Department of Internal Medicine, Okayama University School of Medicine, Okayama), and Shigetaka Asano, M.D., Ph.D. (Institute of Medical Science, Tokyo University, Tokyo).

*Other participants in the Japan Marrow Donor Program are listed in the Appendix.

antigen), determined by serologic typing, increased the risk of acute graft-versus-host disease (GVHD) and decreased overall survival.⁸⁻¹⁰ Thus, in Japan, Europe,¹¹ and the United States,^{12,13} transplantation of cells from unrelated donors is usually performed with donor–recipient pairs that are serologically identical for HLA-A, B, and DR antigens.

The multigene HLA family has at least 12 polymorphic loci: HLA-A, B, C, DRA1, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1. There is strong linkage disequilibrium among the alleles of HLA loci. DNA-amplification methods for typing class I and II HLA alleles have substantiated the extensive polymorphism of the HLA system,¹⁴ initially documented in serologic studies, and have defined alleles that cannot be identified serologically.¹⁵⁻²⁴ Using these molecular techniques, several groups found that matching of DRB1 and DQB1 alleles, but not DPB1 alleles, decreased the risk of acute GVHD and improved survival after the transplantation of hematopoietic stem cells from unrelated donors who were serologically matched with the recipients for HLA-A, B, and DR antigens.²⁵⁻²⁹ The influence of class I HLA alleles was not evaluated in these investigations.

In the present study of stem-cell transplantation in Japan, we performed retrospective DNA typing at 11 polymorphic HLA loci, including class I HLA alleles, in 440 cases of transplantation from unrelated donors who were serologically identical to the recipients with respect to HLA-A, B, and DR antigens. The results showed that genomic matching of class I HLA alleles is an important factor in the clinical outcome of hematopoietic stem-cell transplantation.

METHODS

HLA Typing

Serologic Assays

Serologic typing for HLA-A, B, and DR was performed with use of the standard two-stage complement-dependent test of microtoxicity.³⁰

DNA Amplification

Alleles at the HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1 loci were identified with the use of the polymerase chain reaction (PCR) with sequence-specific oligonucleotide probes,¹⁵⁻²⁰ PCR restriction-fragment–length polymorphisms,^{21,22} PCR single-strand conformation polymorphisms,^{19,23} or PCR sequence-specific primers.²⁴ To verify the reliability of the DNA-based results, we performed HLA genotyping of each sample in two to four laboratories, and resolved by consensus any discrepancies. These HLA-typing results were included in the statistical analyses.

Patients

A total of 440 donor–recipient pairs who were matched serologically for HLA-A, B, and DR antigens participated in this study. Four patients who survived fewer than 10 days after transplantation were excluded from the analysis of acute GVHD. The characteristics of the 440 donor–recipient pairs are summarized in Table 1.

HLA Typing of Patients and Donors

The patients were divided into four groups on the basis of the completeness of HLA genotyping (Table 1). Alleles at 11 polymorphic loci, HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1, were investigated for 363 donor–recipient pairs. HLA-C typing of 84 of the 363 pairs by means of PCR amplification of DNA with the sequence-specific primer could not be achieved. Accordingly, the first 363 pairs were divided into group 1 (279 pairs with HLA-C typing) and group 2 (84 pairs without HLA-C typing). From the analysis of this first set (groups 1 and 2), it was apparent that matching for alleles at HLA-DRB3, DRB4, DRB5, DQA1, DPA1, and DPB1 did not affect the risk of GVHD or survival; therefore, alleles at HLA-A, B, C, DRB1, and DQB1 were examined in the remaining 77 donor–recipient pairs. Information on HLA-C was obtained for 63 pairs but not for the other 14 pairs; thus, group 3 consisted of 63 pairs with HLA-C typing and group 4 of 14 pairs without HLA-C typing.

There were no significant differences among these groups in the base-line characteristics of the donor–recipient pairs (Table 1). Therefore, all the pairs were included in the univariate analysis of the effect of allele matching at the HLA-A, B, DRB1, and DQB1 loci. Groups 1 and 2 were included in the univariate analysis of the effect of allele matching at the HLA-DRB3, DRB4, DRB5, DQA1, DPA1, and DPB1 loci, and groups 1 and 3 were included in the univariate analysis of the effect of allele matching at the HLA-C locus and in the multivariate analysis.

Definition of HLA Mismatching

For each donor–recipient pair we determined whether the mismatch was one in which the recipient's alleles were not shared by the donor (defined as the GVHD vector) or the donor's alleles were not shared by the recipient (defined as the rejection vector). In the analysis of factors contributing to acute GVHD, the GVHD-vector mismatch was used. In the analyses of factors contributing to relapse or death, the mismatch was defined as that of either the GVHD vector or the rejection vector.

Evaluation of GVHD

Occurrences of acute GVHD were graded according to established criteria.^{31,32} The grades were 0, I, II, III and IV, according to the severity of GVHD in the skin, liver, and gastrointestinal tract.

Statistical Analysis

The estimated probability of the development of grade III or IV acute GVHD, the survival rate, and the relapse rate were calculated by the Kaplan–Meier method.³³ The Mantel–Cox test was used to test the equality of cumulative curves for the incidence of grade III or IV GVHD, survival curves, and relapse curves.³⁴ The Cox proportional-hazards model³⁵ was used for multivariate adjustment of various covariates and for quantifying the relations among acute GVHD, death, relapse, and a group of explanatory variables (sex [donor–recipient pair], age of the recipient, age of the donor, diagnosis, leukemia risk group, and treatment) (Table 1). Selection of the factors with an important effect on the rates of acute GVHD, relapse, and survival was based on a forward stepwise procedure.³⁶ P values of 0.05 or less were considered to indicate statistical significance. All statistical analyses were performed with BMDP statistical software (programs 1L, 2L, and 4F).³⁶

RESULTS

Compatibility of HLA Alleles

Among the 440 donor–recipient pairs that were serologically matched for HLA-A, B, and DR antigens, matching with respect to HLA-A, B, DRB1, and DQB1 alleles was found by DNA typing in 73 percent, 84 percent, 82 percent, and 80 percent of the

TABLE 1. BASE-LINE CHARACTERISTICS OF THE DONORS AND RECIPIENTS.*

CHARACTERISTIC	GROUP 1 (N=279)	GROUP 2 (N=84)	GROUP 3 (N=63)	GROUP 4 (N=14)	TOTAL (N=440)
Sex (donor/recipient)					
M/M	100 (36)	24 (29)	23 (37)	8 (57)	155 (35)
M/F	70 (25)	25 (30)	10 (16)	0 (0)	105 (24)
F/F	51 (18)	15 (18)	17 (27)	3 (21)	86 (20)
F/M	58 (21)	20 (24)	13 (21)	3 (21)	94 (21)
Age of recipient (yr)					
1-9	36 (13)	12 (14)	7 (11)	1 (7)	56 (13)
10-19	68 (24)	28 (33)	14 (22)	4 (29)	114 (26)
20-29	79 (28)	24 (29)	16 (25)	5 (36)	124 (28)
30-39	60 (22)	18 (21)	17 (27)	3 (21)	98 (22)
40-50	36 (13)	2 (2)	9 (14)	1 (7)	48 (11)
Age of donor (yr)					
20-29	80 (29)	26 (31)	16 (25)	6 (43)	128 (29)
30-39	111 (40)	34 (40)	26 (41)	5 (36)	176 (40)
40-51	88 (32)	24 (29)	21 (33)	3 (21)	136 (31)
Diagnosis					
Chronic myelogenous leukemia	110 (39)	18 (21)	35 (56)	8 (57)	171 (39)
Acute lymphoblastic leukemia	62 (22)	22 (26)	25 (40)	6 (43)	115 (26)
Acute myeloid leukemia	49 (18)	19 (23)	1 (2)	0	69 (16)
Malignant lymphoma	3 (1)	0	1 (2)	0	4 (1)
Myelodysplastic syndrome	15 (5)	9 (11)	1 (2)	0	25 (6)
Hereditary disease	11 (4)	5 (6)	0	0	16 (4)
Severe aplastic anemia	29 (10)	11 (13)	0	0	40 (9)
Leukemia risk†					
Standard	106 (45)	24 (35)	27 (44)	9 (64)	166 (44)
High	130 (55)	44 (65)	35 (56)	5 (36)	214 (56)
Treatment					
T-cell depletion	6 (2)	2 (2)	2 (3)	0	10 (2)
No T-cell depletion but anti-thymocyte globulin present	45 (16)	19 (23)	9 (14)	2 (14)	75 (17)
No T-cell depletion and no anti-thymocyte globulin	228 (82)	63 (75)	52 (83)	12 (86)	355 (81)

*Donor-recipient pairs were divided into four groups according to the completeness of HLA genotyping (see the Methods section). The median duration of follow-up was 380 days (range, 1 to 1322), and the median age of recipients was 23 years (range, 1 to 50). Because of rounding, not all percentages total 100.

†Patients with a standard risk were defined as those who received a transplant during the first complete remission of acute leukemia or in the first chronic phase of chronic myelogenous leukemia. Patients with a high risk were those with the myelodysplastic syndrome and a more advanced stage of acute leukemia or chronic myelogenous leukemia than those with a standard risk.

pairs, respectively (Table 2). Of these 440 serologically matched pairs, 241 (55 percent) had identical HLA-A, B, and DRB1 alleles. We did not assess matching of HLA-C, DQ, and DP by serologic methods, but we noted in DNA typing that more than 69 percent of the pairs were compatible for alleles at the HLA-C, DQA1, and DQB1 loci. In contrast, fewer than 50 percent of the pairs were compatible with respect to DPA1 and DPB1 alleles.

Acute GVHD

The incidence of acute GVHD of grade III or IV in our study was 18 percent (80 of 436 donor-recipient pairs). In the univariate analysis, no statistically significant associations were observed between the development of grade III or IV acute GVHD and sex (P=0.89), the age of the recipient at the time of transplantation (P=0.84), or the severity of disease (P=0.28).

The occurrence of grade III or IV acute GVHD was significantly associated with disparity in HLA-A, B and C alleles. For HLA-A alleles, the estimated cumulative rate of grade III or IV acute GVHD was 15 percent of the recipients in matched pairs, as compared with 31 percent of mismatched pairs (P<0.001) (Fig. 1A). The corresponding rates for HLA-B were 17 percent and 31 percent (P=0.006), and for HLA-C, 13 percent and 32 percent (P<0.001) (Fig. 1B and 1C). In contrast, matching of DRB1, DQA1, and DQB1 alleles had no significant effect on the occurrence of severe acute GVHD. For DRB1 alleles the rates were 17 percent for matched pairs and 27 percent for mismatched pairs (P=0.058); the rates for DQA1 were 17 percent and 28 percent (P=0.14); and for DQB1 they were 18 percent and 22 percent (P=0.46). The probability that grade III or IV acute GVHD would develop in the recipients in DPA1- and DPB1-matched pairs (19 percent and 18 per-

TABLE 2. EXTENT OF MATCHING OF HLA ALLELES AMONG DONOR-RECIPIENT PAIRS.

HLA Locus*	DONOR-RECIPIENT PAIRS			TOTAL
	TWO HLA MATCHES	ONE HLA MATCH	NO HLA MATCHES	
	number (percent)			number
A	323 (73)	107 (24)	10 (2)	440
B	370 (84)	68 (15)	2 (<1)	440
C	237 (69)	96 (28)	9 (3)	342
DRB1	360 (82)	72 (16)	8 (2)	440
DRB3	354 (98)	0	9 (2)	363
DRB4	363 (100)	0	0	363
DRB5	354 (98)	1 (<1)	8 (2)	363
DQA1	312 (86)	50 (14)	1 (<1)	363
DQB1	353 (80)	80 (18)	7 (2)	440
DPA1	166 (46)	174 (48)	23 (6)	363
DPB1	118 (33)	199 (55)	46 (13)	363

*The total numbers of individual alleles for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, and DPB1 identified in this study by DNA typing were 14, 29, 14, 23, 3, 3, 3, 9, 13, 4, and 14, respectively.

cent, respectively) and mismatched pairs (18 percent and 19 percent, respectively) were almost the same. The probability of grade III or IV acute GVHD among the 160 patients who received hematopoietic stem cells from unrelated donors with whom they were fully matched for HLA-A, B, C, and DRB1 at the allelic level was 11 percent, whereas in a previous study of 120 patients receiving transplants from genotypically identical siblings (matched for HLA-A, B, and DR) it was 6 percent.³⁷

Multivariate analysis revealed that mismatches of the HLA-A allele and the HLA-C allele were significant risk factors for the development of grade III or IV acute GVHD (Table 3). Multivariate estimates of the odds ratios for grade III or IV acute GVHD in pairs matched for the HLA-A alleles or HLA-C alleles were 0.48 (P=0.006) and 0.42 (P=0.001), respectively, as compared with those with mismatches (Table 3). Matching of the HLA-B allele was not a significant factor in the development of acute GVHD (P=0.22). When the HLA-A,C-matched donor-recipient pairs were evaluated for the occurrence of grade III or IV acute GVHD, the probability of its occurrence in 8 pairs mismatched at HLA-B (25 percent) was higher than that in 178 pairs matched at HLA-B (11 percent), although the difference was not statistically significant (P=0.18).

The probability that the recipient in a pair mismatched at the HLA-A, B, or C locus would have grade III or IV acute GVHD was 20 percent when there was one mismatch and 43 percent when there were two or more mismatches, as compared with 11 percent when there were no mismatches (P<0.001).

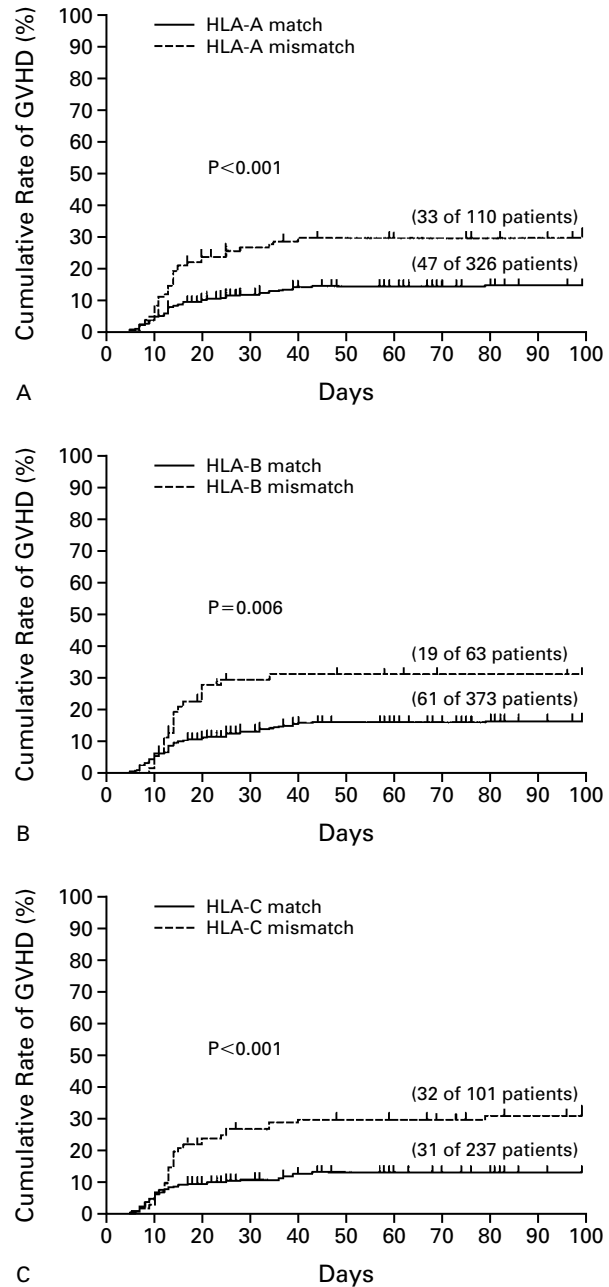


Figure 1. Kaplan-Meier Curves for the Cumulative Probability of Grade III or IV Acute GVHD.

The incidence of GVHD in patients who received transplants matched for the HLA-A, B, or C allele was compared with that in patients who received HLA-A-, B-, or C-mismatched transplants. Results from 436 patients who survived more than 10 days were analyzed. Of these 436 patients, HLA-C alleles were typed for 338. Numbers of patients with GVHD and total numbers for the subgroups are shown in parentheses. Tick marks indicate the patients who died without grade III or IV acute GVHD.

TABLE 3. FACTORS AFFECTING THE INCIDENCE OF GRADE III OR IV ACUTE GVHD, MORTALITY, AND RELAPSE IN RECIPIENTS OF TRANSPLANTS FROM UNRELATED DONORS WHO WERE SEROLOGICALLY MATCHED FOR HLA-A, B, AND DR.*

OUTCOME AND RELEVANT FACTOR	ODDS RATIO (95% CI)	P VALUE
Grade III or IV acute GVHD		
HLA-C allele (match vs. mismatch)	0.42 (0.26–0.70)	0.001
HLA-A allele (match vs. mismatch)	0.48 (0.29–0.79)	0.006
Mortality		
HLA-A allele (match vs. mismatch)	0.45 (0.32–0.62)	<0.001
Age of recipient (≥ 40 vs. < 40 yr)	2.33 (1.59–3.42)	<0.001
Leukemia risk (high vs. standard or none)†	1.69 (1.24–2.29)	<0.001
Diagnosis (AML vs. other diagnoses)	0.63 (0.40–1.01)	0.042
Relapse		
Leukemia risk (high vs. standard)†	3.23 (1.63–6.37)	<0.001
HLA-C allele (match vs. mismatch)	2.22 (0.99–4.97)	0.035

*The variables entered in each analysis were sex (donor–recipient pair), the age of the recipient, the age of the donor, diagnosis, disease risk, and treatment (the characteristics listed in Table 1) and matching of the HLA-A, B, C, DRB1, and DQB1 alleles. CI denotes confidence interval, and AML acute myeloid leukemia.

†Patients with a standard risk were defined as those who received a transplant during the first complete remission of acute leukemia or in the first chronic phase of chronic myelogenous leukemia. Patients with a high risk were those with the myelodysplastic syndrome and a more advanced stage of acute leukemia or chronic myelogenous leukemia than those with a standard risk.

Survival

As shown in Figure 2A, matching of the HLA-A allele was associated with increased survival (survival rate at one year, 63 percent among matched and 38 percent among mismatched recipients; $P < 0.001$). There was a weak association between HLA-B matching and an increased rate of survival (survival rate at one year, 58 percent among matched and 44 percent among mismatched pairs; $P = 0.039$) (Fig. 2B). Even in HLA-A–matched pairs, HLA-B matching was associated with an increased rate of survival among recipients (survival rate at one year, 65 percent among matched and 49 percent among mismatched pairs; $P = 0.045$) (Fig. 2D). Surprisingly, HLA-C matching was not associated with increased survival (survival rate at one year, 57 percent among matched and 53 percent among mismatched pairs; $P = 0.69$), even though mismatching at HLA-C was found to be an important risk factor for acute GVHD (Fig. 2C). Survival rates at one year among recipients matched for the HLA-DRB1, DQA1, and DQB1 alleles were 59 percent, 58 percent, and 59 percent, respectively, as compared with 45 percent, 41 percent, and 42 percent among mismatched recipients ($P = 0.023$, $P = 0.077$, and $P = 0.016$, respectively).

To test whether the effects of class II HLA matching on survival are due to linkage disequilibrium within the HLA system, we analyzed the effect of matching for class II HLA alleles on the survival rate

in the 276 patients matched with their donors for both the HLA-A and the HLA-B allele. In this group, matching or mismatching of the HLA-DRB1, DQA1, or DQB1 alleles had no influence on survival ($P = 0.44$, $P = 0.35$, and $P = 0.080$, respectively) (Fig. 2E and 2F). The survival curves for patients matched for HLA-DPA1 and DPB1 alleles among 203 patients who were also matched for HLA-A, B, DRB1, DRB3, DRB4, and DRB5 alleles did not differ significantly (survival rates at one year, 55 percent and 57 percent, respectively) from the survival curves of DPA1- and DPB1-mismatched patients (survival rates at one year, 57 percent and 55 percent, respectively). The survival rate at one year among the 157 patients who received hematopoietic stem cells from unrelated donors fully matched at HLA-A, B, C, and DRB1 alleles was 65 percent.

Survival was also related to the recipient's age (survival rates at one year, 62 percent for recipients younger than 18 years, 58 percent for recipients 18 to 35 years old, and 41 percent for those older than 35 years; $P = 0.003$) and to the leukemia risk group (survival at one year, 65 percent for those at standard risk and 46 percent for those at high risk; $P < 0.001$), but it was not associated with sex ($P = 0.85$).

Multivariate analysis identified four factors — namely, HLA-A matching, the recipient's age, the leukemia risk group, and diagnosis — as important independent factors affecting mortality among patients receiving hematopoietic stem cells from unrelated donors. Multivariate estimates of the odds ratios for death in recipients with an HLA-A–allele match, an age greater than 40 years, a high risk of leukemia, and a diagnosis of acute myeloid leukemia were 0.45 ($P < 0.001$), 2.33 ($P < 0.001$), 1.69 ($P < 0.001$), and 0.63 ($P = 0.042$), respectively, as compared with recipients without these factors (Table 3).

Relapse of Leukemia

By univariate analysis, the rate of relapse of leukemia in the HLA-C–matched group was higher than that in the HLA-C–mismatched group, although this difference did not reach statistical significance (relapse rate at one year, 23 percent for the matched group and 12 percent for the mismatched group; $P = 0.060$) (Fig. 3). Matches for HLA-A, B (Fig. 3), DRB1, DQA1, DQB1, DPA1, or DPB1 (data not shown) had no influence on the rate of leukemic relapse. Multivariate estimates of the odds ratios for relapse associated with a high risk of leukemia and an HLA-C match were 3.23 ($P < 0.001$) and 2.22 ($P = 0.035$), respectively (Table 3), suggesting that HLA-C matching is a risk factor for leukemic relapse. The relapse rate at one year for the 141 patients who received hematopoietic stem cells from an unrelated donor who was fully matched at the allelic level for HLA-A, B, C, and DRB1 was 20 percent.

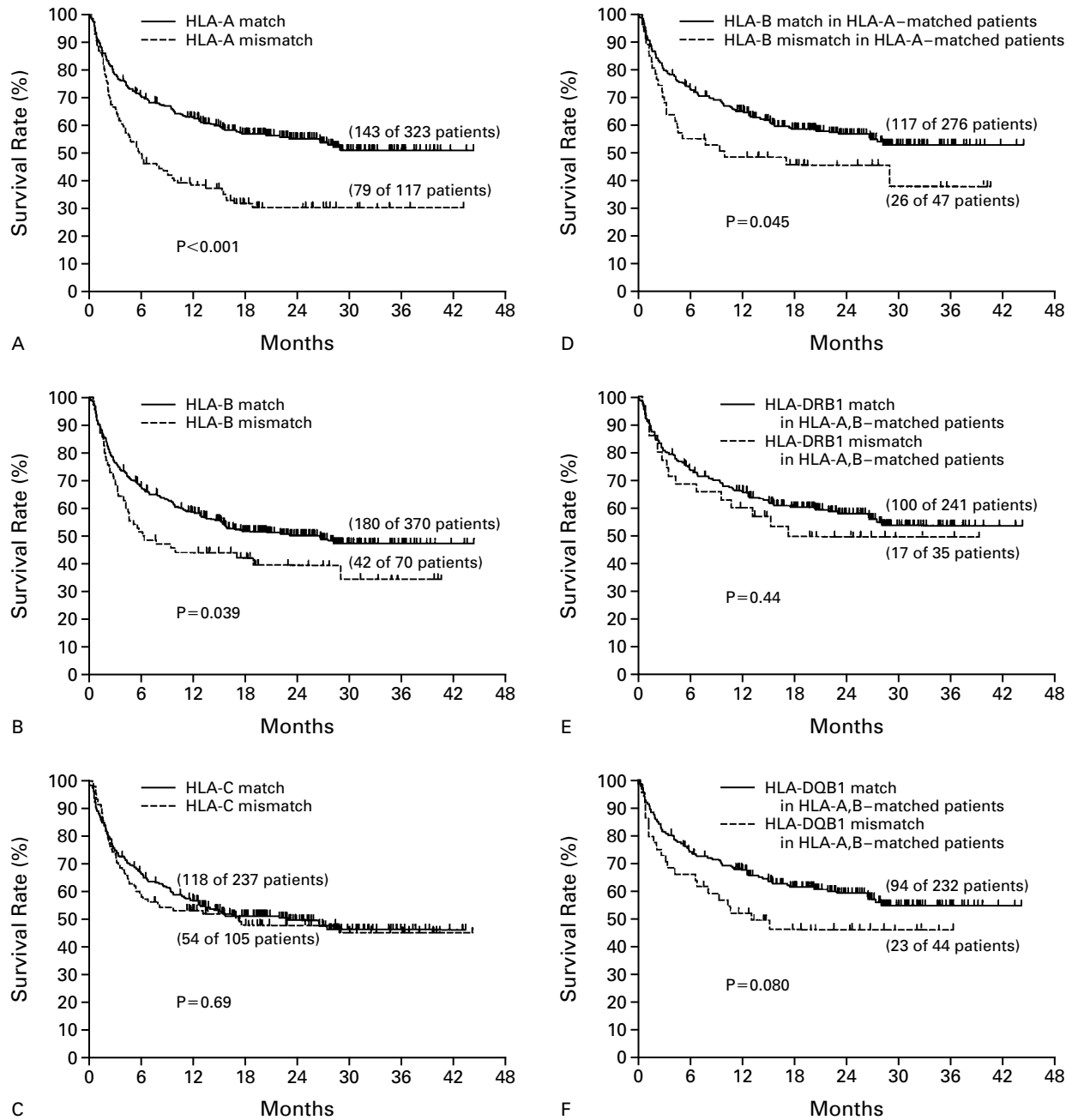


Figure 2. Kaplan–Meier Curves for the Probability of Survival.

The survival of patients who received transplants matched for the HLA-A, B, or C allele was compared with that of patients who received HLA-A-, B-, or C-mismatched transplants (Panels A, B, and C). Survival was also compared between HLA-A-matched patients who received HLA-B-matched transplants and HLA-A-matched patients who received HLA-B-mismatched transplants (Panel D) and between HLA-A,B-matched patients who received HLA-DRB1- or DQB1-matched transplants and HLA-A,B-matched patients who received HLA-DRB1- or DQB1-mismatched transplants (Panels E and F). Numbers of patients who died and total numbers for the subgroups are shown in parentheses. Tick marks indicate the patients who were alive at the time of last contact.

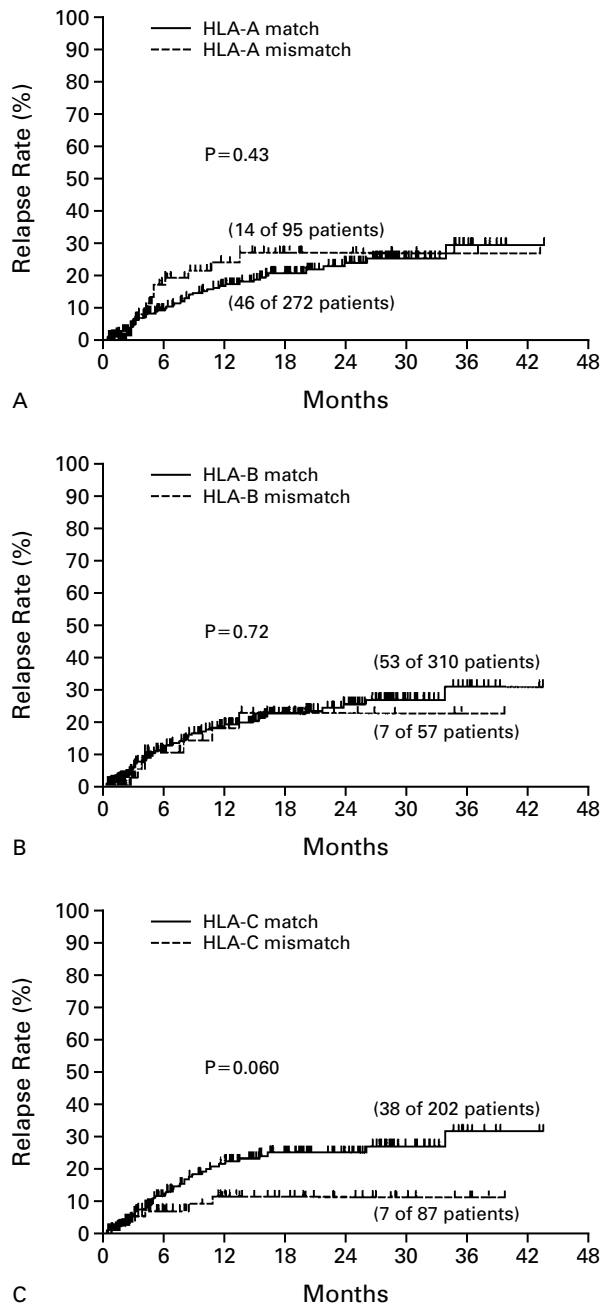


Figure 3. Kaplan–Meier Curves for the Probability of Relapse of Leukemia.

The rate of relapse of leukemia in patients matched for the HLA-A, B, or C allele was compared with that in patients mismatched for HLA-A, B, or C. Among 384 patients with malignant hematologic diseases, relapse data were available for 367. Of these 367 patients, HLA-C alleles were typed for 289. Numbers of patients who had relapses and total numbers for the subgroups are shown in parentheses. Tick marks indicate the patients who were alive at the time of last contact or who died without relapse.

DISCUSSION

Our results show that mismatches with respect to HLA-A and HLA-C alleles between the donor and recipient of hematopoietic stem cells are strong risk factors for the development of grade III or IV acute GVHD in the recipient. By contrast, incompatibilities for class II HLA alleles did not have a critical effect on the risk of acute GVHD. Previous investigations demonstrated that matching of the HLA-DRB1 and DQB1 alleles affects the development of acute GVHD,^{27,28} but the effects of matching of the HLA-A, B, and C alleles were not examined in those studies. Therefore, it is possible that the finding of an association between an increased risk of GVHD and mismatches of alleles at the DRB1 and DQB1 loci actually reflected class I HLA disparities, because of the strong linkage disequilibrium between class I and class II HLA alleles. Alternatively, it is possible that the roles of the individual HLA loci in hematopoietic stem-cell transplantation differ according to ethnic background. Mismatching at the HLA-A locus was observed most frequently for A2 among our Japanese donor–recipient pairs (96 of 190 Japanese patients [51 percent]) whereas in most whites the HLA-A2 allele is A*0201.³⁸ Thus, mismatching for HLA-A2 alleles may be less frequent in white than in Japanese populations.

Previous studies demonstrated that the occurrence of GVHD correlates with a decreased risk of relapse of leukemia after hematopoietic stem-cell transplantation, probably because of T cells or natural killer cells in the graft that are cytotoxic to leukemic cells (a graft-versus-leukemia mechanism).^{39–41} The association we observed between matching of the HLA-C allele and an increased risk of leukemic relapse suggests that the use of stem cells that are mismatched at the HLA-C locus can avert relapse, possibly by inducing a graft-versus-leukemia reaction. Studies of killer-cell inhibitory receptors on natural killer cells and some CD8+ cytotoxic T lymphocytes have shown that specific recognition of HLA-C molecules on target cells by these inhibitory receptors inhibits the lytic activity of the natural killer cells and cytotoxic T cells.^{42–44} Therefore, in principle, a mismatch at HLA-C would allow engrafted natural killer cells and cytotoxic T cells to lyse the recipient’s leukemic cells without the usual inhibition.

The overall rate of survival was significantly affected by HLA-A mismatching, which agrees with our finding that HLA-A mismatching is a strong risk factor for acute GVHD. By contrast, the overall survival rate was not affected by HLA-C incompatibility, even though HLA-C disparity is a strong risk factor for grades III and IV acute GVHD. This discrepancy may be due to the protection from leukemic relapse that is apparently provided by an HLA-C mismatch.

In a univariate analysis, there was a significant difference in the frequency of acute GVHD between

HLA-B-matched and HLA-B-mismatched pairs ($P=0.006$) and a lower rate of survival among HLA-B-mismatched patients, even in the HLA-A-matched group ($P=0.045$). However, in a multivariate analysis we did not find that mismatching for HLA-B alleles was a significant risk factor for acute GVHD and death from any cause. This difference may reflect the relatively small number of allele mismatches at the HLA-B locus, as compared with those at the HLA-A and C loci. For this reason, we believe that HLA-B disparity should not be disregarded as a factor affecting both the risk of GVHD and overall survival.

As for the HLA-DRB1 and DQB1 loci, we found a slightly lower rate of survival in the mismatched group than in the matched group when results in HLA-A-matched and HLA-B-matched patients were evaluated, but the difference did not reach statistical significance. Further investigation is needed to determine the effect of the matching of HLA-DRB1 and DQB1 alleles on survival.

It is very difficult to match HLA-DP alleles together with HLA-A and B alleles because of the lack of strong linkage disequilibrium between these alleles. Therefore, it is fortunate that disparity of HLA-DP had no important effects on the clinical outcome of recipients of hematopoietic stem cells from unrelated donors.

In conclusion, our new, clinically relevant observations are that HLA-A and C disparities at the allelic level are important risk factors for severe acute GVHD; that HLA-A disparity is an important risk factor for death from all causes; that HLA-C matching at the allelic level is a risk factor for relapse of leukemia; and that HLA-B matching at the allelic level is also probably a risk factor both for severe acute GVHD and for death from all causes.

Supported by research funds for bone marrow transplantation from the Ministry of Health and Welfare of Japan.

APPENDIX

The following centers and investigators, all in Japan, participated in the study: *Kyushu University* — S. Yasunaga, S. Yoshitake, T. Koyanagi; *Tokyo Medical and Dental University* — Y. Date; *Japanese Red Cross Central Blood Center* — Y. Ishikawa, K. Sawanaka, K. Kashiwase, A. Ogawa; *Tokai University School of Medicine* — T. Naruse, H. Ando, Y. Matsuzawa; *Chugai Diagnostics Science* — K. Matsubara, A. Kobayashi; *Transplantation centers* — Hokkaido University Hospital, Sapporo University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Hirosaki University Hospital, Tohoku University Hospital, Yamagata University Hospital, Akita University Hospital, Fukushima Medical College, National Cancer Center Central Hospital, Institute of Medical Science at the University of Tokyo, Toho University Hospital, Omori Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Hospital, Itabashi Hospital, Jikei University Hospital, Keio University Hospital, Tokyo Medical College Hospital, Tokyo Medical and Dental University Hospital Faculty of Medicine, Yokohama City University Hospital, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, St. Marianna University Hospital, Chiba Children's Hospital, Matsudo Municipal Hospital, Kameda General Hospital, Saitama Children's Medical Center, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Ibaraki Children's Hospital, Jichi Medical School Hospital, Dokkyo University Hospital, Fukaya Red Cross Hospital, Saiseikai Maebashi Hospital, Gunma

University School of Medicine, Niigata University Hospital, Niigata Cancer Center Hospital, Shinshu University Hospital, Saku Central Hospital, Hamamatsu University Hospital, Hamamatsu Medical Center, Shizuoka General Hospital, Shizuoka Children's Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Nagoya Ekisaikai Hospital, National Nagoya Hospital, Aichi Medical School Hospital, Nagoya City University Hospital, Showa Hospital, Anjo Kousei Hospital, Fujita Health University Hospital, Mie University Hospital, Kanazawa University Hospital, Kanazawa Medical University Hospital, Toyama Prefectural Central Hospital, Fukui Medical School Hospital, Shiga University of Medical Science, Center for Adult Disease in Osaka, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kobe City General Hospital, Kobe University Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Yamaguchi University Hospital, Ehime Prefectural Central Hospital, Okayama National Hospital, Kurashiki Central Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St. Mary's Hospital, Kokura Memorial Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Miyazaki Prefectural Hospital, Kumamoto National Hospital, Kumamoto University Hospital, Oita Medical University Hospital, and Kagoshima University Hospital.

REFERENCES

1. Thomas ED. Marrow transplantation for malignant diseases. *J Clin Oncol* 1983;1:517-31.
2. O'Reilly RJ. Allogeneic bone marrow transplantation: current status and future directions. *Blood* 1983;62:941-64.
3. Champlin RE, Gale RP. Role of bone marrow transplantation in the treatment of hematologic malignancies and solid tumors: critical review of syngeneic, autologous, and allogeneic transplants. *Cancer Treat Rep* 1984; 68:145-61.
4. Bortin MM, Horowitz MM, Gale RP, et al. Changing trends in allogeneic bone marrow transplantation for leukemia in the 1980s. *JAMA* 1992; 268:607-12.
5. Hirabayashi N, Kodera Y, Matsuyama T, et al. Bone marrow transplantation in 614 patients: twenty year experience of the Nagoya Bone Marrow Transplantation Group (NBMTG). *Transplant Proc* 1995;27:1380-2.
6. Hansen JA, Petersdorf E, Martin PJ, Anasetti C. Hematopoietic stem cell transplants from unrelated donors. *Immunol Rev* 1997;157:141-51.
7. Dupont B. Immunology of hematopoietic stem cell transplantation: a brief review of its history. *Immunol Rev* 1997;157:5-12.
8. Beatty PG, Anasetti C, Hansen JA, et al. Marrow transplantation from unrelated donors for treatment of hematologic malignancies: effect of mismatching for one HLA locus. *Blood* 1993;81:249-53.
9. Davies SM, Shu XO, Blazar BR, et al. Unrelated donor bone marrow transplantation: influence of HLA A and B incompatibility on outcome. *Blood* 1995;86:1636-42.
10. Spencer AP, Brookes PA, Kaminski E, et al. Cytotoxic T lymphocyte precursor frequency analyses in bone marrow transplantation with volunteer unrelated donors: value in donor selection. *Transplantation* 1995;59: 1302-8.
11. Hows JM, Yin JL, Marsh J, et al. Histocompatible unrelated volunteer donors compared with HLA nonidentical family donors in marrow transplantation for aplastic anemia and leukemia. *Blood* 1986;68:1322-8.
12. McCullough J, Rogers G, Dahl R, et al. Development and operation of a program to obtain volunteer bone marrow donors unrelated to the patient. *Transfusion* 1986;26:315-23.
13. McElligott MC, Menitove JE, Aster RH. Recruitment of unrelated persons as bone marrow donors: a preliminary experience. *Transfusion* 1986;26:309-14.
14. Bodmer JG, Marsh SG, Albert ED, et al. Nomenclature for factors of the HLA system, 1996. *Tissue Antigens* 1997;49:297-321.
15. Kimura A, Dong RP, Harada H, Sasazuki T. DNA typing of HLA class II genes in B-lymphoblastoid cell lines homozygous for HLA. *Tissue Antigens* 1992;40:5-12.
16. Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991: proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol. 1. Oxford, England: Oxford University Press, 1992:397-419.
17. Date Y, Kimura A, Kato H, Sasazuki T. DNA typing of HLA-A gene: population study and identification of four new alleles in Japanese. *Tissue Antigens* 1996;47:93-101.

18. Yasunaga S, Kimura A, Hamaguchi K, Rønningen KS, Sasazuki T. Different contribution of HLA-DR and DQ genes in susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). *Tissue Antigens* 1996;47:37-48.
19. Tokunaga K, Ishikawa Y, Ogawa A, et al. Sequence-based association analysis of HLA class I and II alleles in Japanese supports conservation of common haplotypes. *Immunogenetics* 1997;46:199-205.
20. Matsubara K, Koide Y, Kobayashi A, et al. A rapid and sensitive method of HLA-DRB1 typing by acridinium-ester-labeled DNA probes. *Hum Immunol* 1992;35:132-9.
21. Inoko H, Ota M. PCR-RFLP. In: Hui KM, Bidwell JL, eds. *Handbook of HLA typing techniques*. Boca Raton, Fla.: CRC Press, 1993:9-70.
22. Naruse TK, Ando R, Nose Y, et al. HLA-DRB4 genotyping by PCR-RFLP: diversity in the associations between HLA-DRB4 and DRB1 alleles. *Tissue Antigens* 1997;49:152-9.
23. Kimura A, Hoshino S, Yoshida M, Harada H, Sasazuki T. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of HLA genes: a practical HLA-matching system. In: Tsuji K, Aizawa M, Sasazuki T, eds. *HLA 1991: proceedings of the Eleventh International Histocompatibility Workshop and Conference*. Vol. 1. Oxford, England: Oxford University Press, 1992:511-4.
24. Ando H, Mizuki N, Ando R, et al. HLA-C genotyping in the Japanese population by the PCR-SSP method. *Tissue Antigens* 1996;48:55-8.
25. Speiser DE, Tiercy JM, Rufer N, et al. High resolution HLA matching associated with decreased mortality after unrelated bone marrow transplantation. *Blood* 1996;87:4455-62.
26. Santamaria P, Reinsmoen NL, Lindstrom AL, et al. Frequent HLA class I and DP sequence mismatches in serologically (HLA-A, HLA-B, HLA-DR) and molecularly (HLA-DRB1, HLA-DQA1, HLA-DQB1) HLA-identical unrelated bone marrow transplant pairs. *Blood* 1994;83:280-7. [Erratum, *Blood* 1994;83:3834.]
27. Petersdorf EW, Longton GM, Anasetti C, et al. Definition of HLA-DQ as a transplantation antigen. *Proc Natl Acad Sci U S A* 1996;93:15358-63.
28. Petersdorf EW, Longton GM, Anasetti C, et al. The significance of HLA-DRB1 matching on clinical outcome after HLA-A, B, DR identical unrelated donor marrow transplantation. *Blood* 1995;86:1606-13.
29. Petersdorf EW, Smith AG, Mickelson EM, et al. The role of HLA-DPB1 disparity in the development of acute graft-versus-host disease following unrelated donor marrow transplantation. *Blood* 1993;81:1923-32.
30. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964;204:998-1000.
31. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation* 1974;18:295-304.
32. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med* 1993;328:593-602.
33. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
34. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966;50:163-70.
35. Cox DR. Regression models and life-tables. *J R Stat Soc [B]* 1972;34:187-220.
36. Dixon WJ, ed. *BMDP statistical software manual*. 3rd ed. Berkeley: University of California Press, 1992.
37. Morishima Y, Morishita Y, Tanimoto M, et al. Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings: possible role of genetic homogeneity. *Blood* 1989;74:2252-6.
38. Fleischhauer K, Zino E, Mazzi B, Severini GM, Benazzi E, Bordignon C. HLA-A02 subtype distribution in Caucasians from northern Italy: identification of A0220. *Tissue Antigens* 1996;48:673-9. [Erratum, *Tissue Antigens* 1997;50:208.]
39. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979;300:1068-73.
40. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990;75:555-62.
41. Hauch M, Gazzola MV, Small T, et al. Anti-leukemia potential of interleukin-2 activated natural killer cells after bone marrow transplantation for chronic myelogenous leukemia. *Blood* 1990;75:2250-62.
42. Moretta A, Biassoni R, Bottino C, et al. Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev* 1997;155:105-17.
43. Reyburn H, Mandelboim O, Vales-Gomez M, et al. Human NK cells: their ligands, receptors and functions. *Immunol Rev* 1997;155:119-25.
44. Moretta A, Moretta L. HLA class I specific inhibitory receptors. *Curr Opin Immunol* 1997;9:694-701.

CORRECTION

Effect of Matching of Class I HLA Alleles on Clinical Outcome after Transplantation of Hematopoietic Stem Cells from an Unrelated Donor

Effect of Matching of Class I HLA Alleles on Clinical Outcome after Transplantation of Hematopoietic Stem Cells from an Unrelated Donor . On page 1177, the sentence that starts on line 7 of the Results paragraph of the Abstract should have read, "*Matching* of HLA-C alleles was a significant risk factor for relapse of leukemia," not "*Mismatching* of HLA-C alleles," as printed. We regret the error.