the pharmacokinetic profile of SR29142 for 10 patients in each dose group, assessment of anti-SR29142 antibody production and estimation of the optimal dosage of SR29142 for use in Japanese patients. The study was approved by the institutional review boards of all participating institutions. Written informed consent was obtained from all participants before randomization.

A total of 50 Japanese adult patients were recruited and received SR29142 administered at one of two dose levels: 0.15 or 0.20 mg/kg per day. The pharmacokinetics study was conducted in the first 10 patients at each dose level. The safety and efficacy evaluation board reviewed the safety profile of these first 20 patients in order to determine whether the study could be continued.

Patients were classified as being either at high risk or at potential risk of developing TLS-associated hyperuricemia. High risk was defined as: hyperuricemia of malignancy (plasma uric acid, >7.5 mg/dL); very aggressive lymphoma/leukemia according to the Proposed Clinical Schema for Malignancies of the Lymphoid System[8] based on the Revised European-American Lymphoma (REAL);[9] acute myelocytic leukemia; chronic myelocytic leukemia in blast crisis; or high-grade myelodysplastic syndrome with 10% bone marrow blast involvement and undergoing aggressive treatment similar to acute myelocytic leukemia. Potential risk was defined as aggressive lymphoma/leukemia[8] plus one or more of the following criteria: lactate dehydrogenase of twice the upper limit of normal; stage III/IV disease; or stage I/II disease with one lymph node or tumor of more than 5 cm in diameter. In all cases, vital signs were monitored just before administration, then at 10–20 and 30–40 min after initiation of each administration. Standard laboratory tests were performed at baseline, and on days 1, 3, 5, 8, 15, 22, 29 and 36. Creatinine clearance was evaluated on days 1 and 8.

Patient eligibility. To be eligible for the study adult patients (aged 18–74 years) scheduled for chemotherapy for leukemia or lymphoma needed to meet at least one of the following criteria: acute leukemia with a white blood cell count of 20 000/μL or more; stage III/IV malignant lymphoma (not further specified) regardless of plasma uric acid level; stage II malignant lymphoma with bulky disease (defined as a node or nodal mass ≥10 cm, or with maximum mediastinal mass width at least one-third of the internal transverse diameter of the thorax at the level of T5/6); any leukemia or lymphoma associated with plasma uric acid of 8.0 mg/dL or more and lactate dehydrogenase twice the upper limit of normal. These patients were considered to have potential or a high risk of TLS. A performance status of 3 or less on the Eastern Cooperative Oncology Group scale and an estimated life expectancy of at least 40 days were also required.

Patients were excluded if: they received allopurinol within 72 h prior to the start of SR29142; were scheduled to receive asparaginase; had a known history of significant allergic reactions; had a documented history of asthma or asthmatic bronchitis; were glucose-6-phosphate dehydrogenase deficient; or were pregnant or lactating women.

Treatment modalities. SR29142 (0.15 or 0.20 mg/kg) was administered once daily for 5 consecutive days by i.v. infusion over 30 min. The dosing schedule of 0.15 or 0.20 mg/kg was randomly allocated based on the stratification of underlying disease (lymphoma or acute leukemia) and uric acid level (8 mg/dL vs <8 mg/dL). The drug infusion was started at the same time on day 1 through to day 5. Chemotherapy for lymphoma or leukemia was started within 4–24 h after the first dose of SR29142. Between the start of SR29142 administration and the start of chemotherapy, neither prophylactic treatment with antiemetic drugs nor treatment with sodium bicarbonate for alkalinization of the urine was permitted.

Efficacy evaluation. The primary efficacy end-point was overall response rate (ORR) for SR29142 treatment, defined as the normalization of uric acid levels as determined by assays of plasma uric acid concentration. Treatment was considered to be successful and the patient considered to be a treatment responder if the plasma uric acid level had decreased to 7.5 mg/dL or less 48 h after the start of the first SR29142 infusion and was maintained until 24 h after the start of the final (day 5) SR29142 infusion. Patients who failed to complete 5 days of treatment were classified as non-responders, even if their uric acid levels were normal.

Secondary end-points included the rate of plasma uric acid concentration decline over time following the first administration of SR29142, urinary allantoin levels and excretion rate, and renal function (serum creatinine, creatinine clearance, potassium, and phosphorus or calcium levels). Plasma uric acid levels were determined from blood samples collected via polypropylene tubes containing anticoagulant (heparin). To prevent the enzymatic action of SR29142, samples were placed on ice immediately after collection, centrifuged and frozen until measurement. The standard method used at each institution was performed to determine plasma uric acid. Plasma uric acid sampling was performed on: day 1 (just before treatment administration; 4 and 8 h after starting administration); days 2, 3 and 4 (just before treatment administration); day 5 (just before treatment administration; 8 h after starting administration); day 6 (24 h after starting administration on day 5); day 8 (72 h after starting administration on day 5); and day 15. Other hyperuricemia agents, such as allopurinol, were not to be used until after blood sampling for uric acid assay on day 15. The *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5dimethoxy-4-fluoranilline and the peroxidase assay methods were used for determination of plasma uric acid concentrations at each institution. Urinary allantoin concentrations were determined by an electrospray ionization liquid chromatography with tandem mass spectrometry method with a limit of quantification of 13.6 mg/mL. Urinary allantoin sampling was performed 24 h before initial drug administration and on days 1–7. SR29142 plasma concentration was determined by a validated immunoenzymometric assay with a limit of quantification of 0.7 ng/mL.

Safety evaluation. Safety assessments were based on clinical observation, standard laboratory tests, vital signs (blood pressure, pulse and body temperature) and the occurrence of AE. The severity of AE was graded according to the National Cancer Institute–Common Toxicity Criteria version 2.0. All AE that occurred during SR29142 monotherapy and during the administration of concomitant chemotherapy were to be recorded on Case Report Forms by the investigator. The relationship (related/not related) of AE to SR29142 was assessed by the individual investigators. Antibody measurement was performed on days 1, 8 and 29 in all patients. Further measurements were conducted in patients who tested positive. Levels of human immunoglobulin (hIg) anti-SR29142 antibodies from blood samples were determined by enzyme-linked immunosorbent assay using the following method. All wells of the microplates were coated with SR29142. Standard and circulating hIg were immunofixed to the coated SR29142 standard and were detected with anti-hIg–peroxidase conjugate. The peroxidase activity was detected with a chromogenic substrate (O-phenylene-diamine); and absorption at 492 nm correlated to the amount of anti-SR29142 antibodies in the plasma.[10]

Statistical analysis. All patients who received at least one dose of SR29142 and had at least one post-baseline efficacy evaluation were included in the efficacy population. All patients who received at least one dose of SR29142 were included in the safety population. Descriptive statistics are provided throughout using an observed-cases approach. *P*-values (two-tailed Student's *t*-test) and 95% confidence intervals (CI) were calculated, with $P < 0.05$ regarded as significant.

Assuming that the true response rate would be 95%, the probability of observing at least 23 responders among 25 patients treated with each dose of SR29142 would be 87.3%. If 23

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Table 1. Baseline characteristics of 50 eligible patients

	SR29142		Total
	0.15 mg/kg (n = 25)	0.20 mg/kg (n = 25)	(n = 50)
Age (years)			
Median	51	55	54
Range	19–73	23–73	19–73
Age class, n (%)			
<65 years	20 (80)	18 (72)	38 (76)
≥65 years	5 (20)	7 (28)	12 (24)
Sex, n (%)			
Male	11 (44)	13 (52)	24 (48)
Female	14 (56)	12 (48)	26 (52)
ECOG performance status, n (%)			
0	17 (68)	17 (68)	34 (68)
1	3 (12)	4 (16)	7 (14)
2	5 (20)	2 (8)	7 (14)
3	0 (0)	2 (8)	2 (4)
Diagnosis, n (%)			
Lymphoma	21 (84)	21 (84)	42 (84)
Stage II	2 (8)	2 (8)	4 (8)
Stage III	6 (24)	8 (32)	14 (28)
Stage IV	13 (52)	11 (44)	24 (48)
Acute lymphocytic leukemia	2 (8)	0 (0)	2 (4)
Acute myelogenous leukemia	2 (8)	4 (16)	6 (12)
Hyperuricemic at baseline (≥8 mg/dL), n (%)			
Yes	3 (12)	2 (8)	5 (10)
No	22 (88)	23 (92)	45 (90)
Risk category, n (%)			
High	6 (24)	7 (28)	13 (26)
Potential	19 (76)	18 (72)	37 (74)

ECOG, Eastern Cooperative Oncology Group.

responders were observed, the 95% two-sided confidence lower limit for the response rate would be 74.0%. It could therefore be concluded with 97.5% confidence that the true response rate would be at least 74.0%. In line with clinical findings showing that any anti-SR29142 antibodies are usually produced within a month of treatment, only patients who had tested positive for the presence of anti-SR29142 antibodies on day 29 were to be followed up after the study period.

Dose proportionality for area under concentration–time curve from 0–24 h (AUC_{0-24}) and plasma concentration at the end of infusion (C_{eoi}) were evaluated using the log-transformed power model. An estimate and 90% CI for the difference in dose group means were computed within the mixed-model framework and converted to a ratio of adjusted means by the anti-log transformation. Within-patient, between-patient and total variances were estimated for log AUC_{0-24} by equating observed and expected mean squares within the linear mixed-effects model described above. The 95% CI for the variances were computed using the simple χ^2-test method for within-patient variance, the Modified Large Sample procedure for between-patient variance, and the Graybill–Wang procedure for total-patient variance. Variance estimates were expressed as standard deviations. All analyzes were carried out by using SAS ver. 8.2 (SAS Institute, Cary, NC, USA).

Results

Patients. Between April 2003 and June 2004, 50 adult Japanese patients with leukemia and/or lymphoma were enrolled in this study from nine centers. Demographic and baseline characteristics are summarized in Table 1. Overall, demographic characteristics were similar between the two dosage groups. A total of five patients (10%) were hyperuricemic at baseline and approximately half (n = 24) had stage IV lymphoma. Thirteen patients (26%) were defined as having a high risk for TLS-associated hyperuricemia and 37 patients (74%) were defined as having a potential risk.

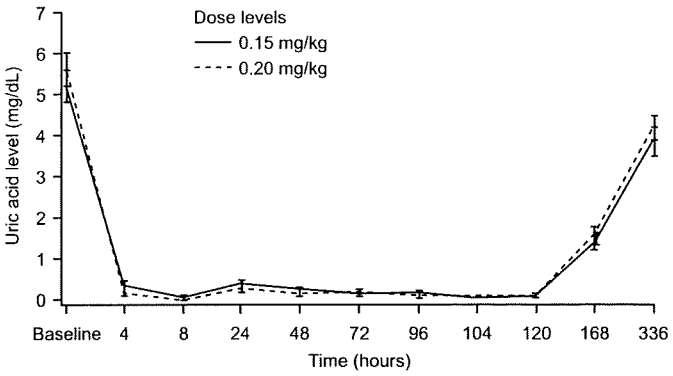

Fig. 1. Mean plasma uric acid concentrations by dose over time. Patients with leukemia or lymphoma were randomly allocated (based on stratification by underlying disease and uric acid level) to receive SR29142 administered at either 0.15 or 0.20 mg/kg per day for 5 days, followed by chemotherapy starting from 4 to 24 h after the first infusion of SR29142.

Drug administration. Forty-nine patients (98%) completed 5 days of treatment. One patient who received the 0.20 mg/kg dose did not complete 5 days of treatment due to a severe AE (elevated liver enzymes).

Control of plasma uric acid and excretion of allantoin. Mean plasma uric acid concentrations of both cohorts over time are presented in Figure 1. Uric acid levels declined immediately after the administration of SR29142 and was maintained below 1 mg/dL at the measurement point (4 h after administration) until 120 h. The uric acid concentration was maintained at low levels thereafter (during the concomitant chemotherapy period) and was normalized by day 15 (Fig. 1).

Mean daily urinary allantoin levels in all patients receiving SR29142 at various time points were as follows: pre-dose, 12.1 mg; day 1, 1280 mg; day 3, 1030 mg; day 5, 897 mg; and day 7368 mg. Compared with background levels, the amounts of allantoin in the urine were increased approximately 100-fold after SR29142 treatment, with no difference between the two dose levels (data not shown).

Efficacy. The ORR was 100.0% (95% CI, 86.3–100.0%) in the 0.15 mg/kg group and 96.0% (95% CI, 79.6–99.9%) in the 0.20 mg/kg group. The total ORR was 98.0% (95% CI, 89.3–100.0%). One patient in the 0.20 mg/kg treatment group was removed from the study due to a severe AE following the investigator's judgment. Although the uric acid level of this patient decreased to less than 0.1 mg/dL for the 3 days during which they had received SR29142, the final uric acid level was not obtained. Therefore the patient was classified as a non-responder as defined in the protocol.

Adverse events. Due to the nature and severity of the underlying illness and concomitant chemotherapy, all patients had at least one AE. AE that occurred in 20% or more of patients during the study were similar to those commonly reported for chemotherapy in patients with lymphoma and leukemia (Table 2a). Drug-related AE judged by the investigators occurred in 23 patients (46%) overall: 10 patients (40%) in the 0.15 mg/kg group and 13 patients (52%) in the 0.20 mg/kg group. The most frequently occurring drug-related AE were elevated liver enzymes (24%).

Table 2a. Adverse events occurring in 20% or more of patients

Adverse event	SR29142			
	0.15 mg/kg (n = 25)		0.20 mg/kg (n = 25)	
	All grades n (%)	Grade 3/4 n (%)	All grades n (%)	Grade 3/4 n (%)
White blood cells decreased	24 (96)	22 (88)	22 (88)	21 (84)
Neutrophil count decreased	22 (88)	22 (88)	19 (76)	19 (76)
Alopecia	20 (80)	0 (0)	18 (72)	0 (0)
Lymphocyte count decreased	16 (64)	15 (60)	18 (72)	15 (60)
Nausea	12 (48)	0 (0)	15 (60)	1 (4)
Constipation	10 (40)	4 (16)	11 (44)	3 (12)
Aspartate aminotransferase increased	6 (24)	0 (0)	11 (44)	1 (4)
Hemoglobin decreased	11 (44.0)	4 (16)	6 (24)	0 (0)
Platelet count decreased	8 (32)	4 (16)	9 (36)	3 (12)
Alanine aminotransferase increased	7 (28)	1 (4)	8 (32)	1 (4)
Anorexia	7 (28)	1 (4)	8 (32)	1 (4)
Malaise	7 (28)	0 (0)	8 (32)	0 (0)
Vomiting	6 (24)	0 (0)	9 (36)	0 (0)
Diarrhea	8 (32)	0 (0)	4 (16)	0 (0)
Hyperglycemia	6 (24)	2 (8)	5 (20)	1 (4)
Pyrexia	5 (20)	0 (0)	6 (24)	1 (4)
Stomatitis	3 (12)	0 (0)	8 (32)	1 (4)
Blood bilirubin increased	7 (28)	0 (0)	3 (12)	0 (0)
Blood lactate dehydrogenase increased	5 (20)	0 (0)	5 (20)	1 (4)

Table 2b. SR29142-related adverse events that occurred before initiation of first chemotherapy

Adverse events	SR29142		Total (n = 50) n (%)
	0.15 mg/kg (n = 25) n (%)	0.20 mg/kg (n = 25) n (%)	
Application site pain	0 (0)	1 (4)	1 (2)
Pyrexia	1 (4)	0 (0)	1 (2)
Hypersensitivity	1 (4)	2 (8)	3 (6)
Anorexia	1 (4)	0 (0)	1 (2)
Rash	0 (0)	1 (4)	1 (2)

Table 3. Production of SR29142 antibodies

Sampling date	SR29142			
	0.15 mg/kg		0.20 mg/kg	
	n	Positive no. (%)	n	Positive no. (%)
Day 1	25	0 (0)	25	0 (0)
Day 8	25	0 (0)	24*	0 (0)
Day 29 (± 2 days)	25	2 (8)	25	3 (12)
Follow-up period				
3 months (± 2 weeks)	2	0 (0)	3	3 (12)
6 months (± 2 weeks)	0	–	3	2 (8)
1 year (± 2 weeks)	0	–	1	0

*One patient was withdrawn from treatment on day 3; this patient was monitored on day 29 but not on day 8.

Given the potential risk of anaphylaxis associated with SR29142, it was decided to evaluate the safety of the drug administered as monotherapy during the 5 consecutive days before chemotherapy. Drug-related AE that occurred before initiation of concomitant chemotherapy are summarized in Table 2(b). Although six patients developed seven drug-related AE before chemotherapy (SR29142-related hypersensitivity reactions occurred in three patients during the SR29142-administration period, and application site pain, pyrexia, anorexia and rash occurred in one patient each), none of these events were categorized as grade 3 or 4 severity and patients recovered immediately.

Overall, during the entire study, hypersensitivity reactions, regardless of relationship to study medication, occurred in 35 patients (70%); eight patients (16%) experienced reactions of grade 3 or 4 severity. Hypersensitivity reactions that were likely to be related to SR29142 occurred in 11 patients (22%): five patients in the 0.15 mg/kg group and six patients in the 0.20 mg/kg group. In two patients, both of whom were in the 0.20 mg/kg group, these events were categorized as grade 3 or 4. All hypersensitivity reactions were manageable and resolved with no sequels.

Three patients had serious AE during the study. One patient treated with 0.15 mg/kg developed unstable angina, and in the 0.20 mg/kg group, one patient had sepsis and septic shock, and one patient had elevated liver enzymes (resulting in study discontinuation). Of these events, only the case of increased liver enzymes was considered to be related to SR29142 following assessment by the Efficacy/Safety Evaluation Committee and the investigator. This patient was a 54-year-old Japanese woman with stage IV follicular lymphoma. Increased liver enzyme levels (grade 3) were noted before drug administration on day 3 and SR29142 was permanently discontinued. Liver enzyme levels were nearly normalized by day 16. No patients experienced hemolysis or methemoglobinemia and no deaths occurred during the study.

Renal outcome. There were no clinically significant changes from baseline during chemotherapy in the renal parameters: creatinine clearance, potassium, phosphorous, and calcium (data not shown).

Production of anti-SR29142 antibodies. The time course of anti-SR29142 antibody production is summarized in Table 3. None of the patients had any anti-SR29142 antibodies on day 8. Five patients (10%) had the antibodies on day 29 (two in the 0.15 mg/kg group and three in the 0.20 mg/kg group); after 6 months, antibodies were only detected in two patients (both in the 0.20 mg/kg group). After 1 year, one patient had no antibodies;

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Table 4. Pharmacokinetic parameters of SR29142

	SR29142†					
	0.15 mg/kg (n = 11)			0.20 mg/kg (n = 10)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Day 1						
AUC_{0-24} (ng.h/mL)	45 653	7544	17	59 333	15 849	27
C_{eoi} (ng/mL)	3734	1081	29	4239	1556	37
Day 5						
AUC_{0-24} (ng.h/mL)	48 210	9660	20	65 154	22 713	35
$t_{½z}$ (h)	22.5	5.8	26	16.1	5.6	35
C_{eoi} (ng/mL)	3948	710	18	5126	1468	29
C_{min} (ng/mL)	852	269	32	1033	494	48

†SR29142 was administered once daily for 5 consecutive days. AUC_{0-24}, area under the concentration–time curve for 0–24 h; C_{eoi}, plasma concentration of end of infusion; C_{min}, minimum plasma concentration; CV, coefficient of variance; SD, standard deviation; $t_{1/2z}$, terminal elimination half-life.

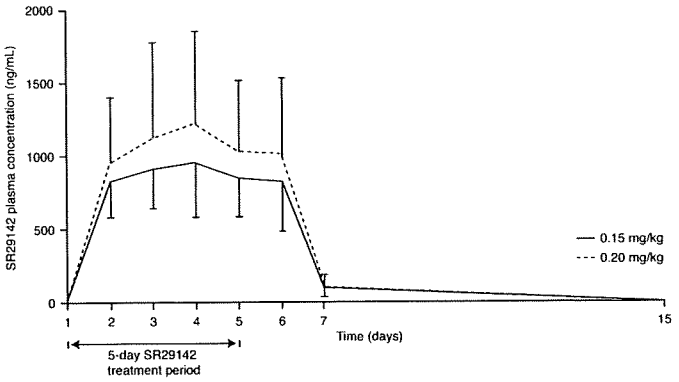

Fig. 2. Mean (standard deviation) SR29142 plasma C_{eoi} concentrations after once-daily repeated 30-min i.v. infusions for a total of 5 days. Patients with leukemia or lymphoma were randomly allocated (based on stratification by underlying disease and uric acid level) to receive SR29142 administered at either 0.15 or 0.20 mg/kg per day for 5 days, followed by chemotherapy starting from 4 to 24 h after the first infusion of SR29142.

the other had been lost to follow up. Of the 11 patients who experienced SR29142-related hypersensitivity reactions during the study, one patient tested positive for anti-SR29142 antibodies. Although hypersensitivity reaction (rash) was observed on day 1, before the start of chemotherapy in this patient, no anti-SR29142 antibodies had been produced in this period.

Pharmacokinetics. The mean SR29142 pharmacokinetics parameters are shown in Table 4. Increased exposure to SR29142, as measured by AUC_{0-24} and C_{eoi}, was dose proportional (Fig. 2). For the 1.33-fold difference in dose between 0.15 and 0.20 mg/kg, the AUC_{0-24} was 1.28-fold and 1.31-fold higher, and C_{eoi} was 1.11-fold and 1.27-fold higher for 0.20 versus 0.15 mg/kg on days 1 and 5, respectively. Steady state was reached between days 2 and 3 and terminal half-life ($t_{½z}$) was comparable for both dose groups. The accumulation ratio of AUC_{0-24} (defined as the ratio of day 5 to 1 AUC_{0-24}) was 1.07 (95% CI, 0.99–1.14), indicating slight accumulation of SR29142.

Discussion

SR29142 is used as a supportive drug in patients with cancer and is administered with concomitant chemotherapeutic agents. This is the first study to evaluate pharmacokinetics and AE related to SR29142 before the initiation of chemotherapy in Japanese patients with hematological malignancies. As expected, the majority of AE can be attributed to the patients' underlying cancer status and/or concomitant cytotoxic drug administration. In this study, six patients had a total of seven AE, including three hypersensitivity reactions, before the first dose of chemotherapy. These toxicities, however, were of grade 1 or 2 severity and were all manageable. After the first dose of chemotherapy, no unexpected SR29142-related AE were observed. These results show that single-agent SR29142 has an associated low-toxicity profile in adult patients with lymphoma and acute leukemia.

Because SR29142 is a recombinant protein that is exogenous to humans, the production of antibodies to this agent is a potential concern, although the clinical significance of the development of anti-SR29142 antibodies remains unknown. In this study, 10% of patients developed anti-SR29142 antibodies in line with previously reported data,[11] and none of the patients had any anti-SR29142 antibodies on day 8. Importantly, of the 11 patients who experienced hypersensitivity reactions likely to be related to SR29142, only one patient tested positive for SR29142-related antibodies. In one patient, although hypersensitivity reaction (rash) was observed on day 1, before the start of chemotherapy, it was confirmed that no anti-SR29142 antibodies were produced during this period.

The concentration of plasma uric acid was controlled rapidly with SR29142 and the ORR was 98%. Additionally, the ability of SR29142 to prevent hyperuricemia was further supported for both doses by the appearance of large amounts of urinary allantoin, the end product of uric acid metabolism by SR29142, and a marker of its activity. The finding that renal function remained stable during the study indicates the ability of SR29142 to indirectly prevent TLS. These results are consistent with those reported in patient populations in European and North American countries.[11–16]

The pharmacokinetic findings of the current study support the premise that SR29142 exerts dose-proportional effects. Furthermore, the accumulation ratio of AUC_{0-24} showed that there was a slight accumulation of SR29142 during the study. These results are comparable with those reported in European and North American populations.[17] Therefore, no ethnic differences are associated with the pharmacokinetics of SR29142.

Both doses (0.15 and 0.20 mg/kg) of SR29142 were safe and effective under the study conditions. No differences in the toxicity or efficacy profiles were observed between the two dose groups. As this is a phase II study, the optimal dose cannot be defined definitely, but previous findings show that 0.20 mg/kg is well tolerated in adult patients with non-Hodgkin's lymphoma.[12] Furthermore, recent guidelines for the management of patients with TLS[18] report that rasburicase 0.20 mg/kg is appropriate for seriously ill patients with baseline hyperuricemia or for those who are at high risk of developing TLS, whereas the 0.15 mg/kg dose should be used in patients without baseline hyperuricemia but who have a potential risk of TLS.

In conclusion, SR29142 is highly effective as a supportive drug during chemotherapy to control hyperuricemia, which can induce TLS in adult patients with malignant lymphoma and acute leukemia. SR29142 was well tolerated, with a good safety profile when administered as a single agent prior to the commencement of chemotherapy.

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ORIGINAL ARTICLE

Busulfex (i.v. BU) and CY regimen before SCT: Japanese-targeted phase II pharmacokinetics combined study

S-W Kim[1], S-i Mori[1], R Tanosaki[1], T Fukuda[1], M Kami[2], H Sakamaki[3], T Yamashita[3], Y Kodera[4], S Terakura[5], S Taniguchi[6], S Miyakoshi[7], N Usui[8], S Yano[8], Y Kawano[9], Y Nagatoshi[10], M Harada[11], Y Morishima[12], S Okamoto[13], AM Saito[14,15], Y Ohashi[15], R Ueda[16] and Y Takaue[1]

[1]*Hematology and Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan;* [2]*Division of Exploratory Research, Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [3]*Department of Hematology, Komagome Hospital, Tokyo, Japan;* [4]*Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [5]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [6]*Department of Hematology, Toranomon Hospital, Tokyo, Japan;* [7]*Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan;* [8]*Division of Clinical Oncology and Hematology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan;* [9]*Department of Pediatrics, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan;* [10]*Section of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan;* [11]*National Omuta Hospital, Omuta, Japan;* [12]*Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan;* [13]*Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan;* [14]*Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [15]*Department of Biostatistics/Epidemiology and Preventive Health Sciences, School of Health Sciences and Nursing, University of Tokyo, Tokyo, Japan and* [16]*Department of Internal Medicine and Molecular Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan*

To evaluate the toxicity and efficacy of an i.v. preparation of BU (12.8 mg/kg), combined with CY (120 mg/kg), a prospective study was performed on 30 Japanese patients (median age, 30 years) with hematologic malignancies undergoing hematopoietic SCT (28 allogeneic transplants from an HLA-matched donor and 2 autologous transplants). There were no significant toxicities, and all but one patient showed evidence of granulocyte engraftment at a median of 14 days for allogeneic and 11 days for autologous transplantation. Grades II–IV acute and chronic GVHD occurred in 9 (9/27, 33%) and 16 patients (16/27, 59%), respectively. Non-relapse mortality at days 100 and 365 was 3 and 17%, respectively. The pharmacokinetics of i.v. BU showed close inter- and intrapatient consistency; the area under the plasma concentration–time curve of the first administration remained at less than 1500 μmol min/l in 27 of the 29 patients (93%), and between 900 and 1350 μmol min/l in 22 patients (73%). As all of the profiles overlap with data from non-Japanese patients, we conclude that racial factors may not seriously influence the bioactivity of i.v. BU.

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Correspondence: Dr Y Takaue, Hematology and Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-Ku Tokyo 104-0045, Japan.
E-mail: ytakaue@ncc.go.jp
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Introduction

In hematopoietic SCT (HSCT), high-dose BU has been widely used, mostly in combination with CY.[1] To overcome the disadvantage of oral BU including gastrointestinal absorption,[2–16] i.v. BU was recently introduced into clinical use.[17–20] The initial experience with i.v. BU showed satisfactory dose assurance with reliable predictability of pharmacokinetics without dose adjustment.[19] Hence, it is very probable that its use reduces the incidence of various risks at transplantation such as hepatic venoocclusive disease (VOD), as shown by Kashyap *et al.*[21]

Nevertheless, drug profiles of i.v. BU preparation have not been fully evaluated in different races, who may have different pharmacokinetics. As part of our pivotal study in Japan, we conducted a phase II study with pharmacokinetic analysis of a combined i.v. BU and CY (BU/CY) regimen administered before allogeneic or autologous HSCT. A population pharmacokinetic analysis suggested that i.v. BU pharmacokinetics show high inter- and intrapatient consistency.[22] This study with the same population further focused on complete pharmacokinetic profiles with additional clinical and safety data.

Patients and methods

Eligibility criteria

Patients with acute leukemia, CML, MDS or malignant lymphoma were eligible for this study. Patients aged 5–55 years with a Lansky Performance Status >70 (over 5 and less than 16 years of age) or an Eastern Cooperative Oncology Group Performance Status ≤2 (16–55 years of

age) who were expected to survive beyond 100 days after HSCT were eligible. The eligibility criteria also included serum creatinine less than twice the upper normal limit, as well as serum total bilirubin less than 1.5 times, and aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltranspeptidase less than three times the upper normal limit. Left ventricular ejection fraction $\geqslant 50\%$ or arterial blood oxygen saturation $\geqslant 94\%$, and in adult patients a carbon monoxide lung diffusing capacity $\geqslant 60\%$, were required. Patients with arrhythmia, hypertension or diabetes mellitus that was difficult to control despite medication, severe cardiopulmonary or renal disease, chronic active hepatitis, liver cirrhosis, acute hepatitis, ascites more than 1 l, central nervous system disorders, active infection; positive hepatitis B surface antigen, hepatitis B core antibody, hepatitis C virus antibody or human immunodeficiency virus antigen/antibody; or prior HSCTs were all excluded. Patients were also required to have either BM available from an HLA-matched related or unrelated donor or G-CSF-mobilized PBSCs available from an HLA-matched related donor without T-cell depletion. The study was conducted in conformity with ICH-GCP and the Declaration of Helsinki. The protocol and informed consent forms were approved by each institution's Research Ethics Committee. All patients gave written informed consent prior to their participation in the study.

Conditioning regimen

The i.v. BU (KRN246; Kirin Pharma Co. Ltd., Tokyo, Japan) was given at 0.8 mg/kg through a central venous catheter for 2 h every 6 h at a total of 16 doses for 4 days on days −7 to −4. CY 60 mg/kg was administered through a central venous catheter for 3 h at a total of two doses for 2 days on days −3 and −2. After a rest on day −1, BM or G-CSF-mobilized PBSC without T-cell depletion was infused on day 0. A fixed-dose regimen for BU was calculated based on either the ideal body weight or actual body weight, whichever was less, for adults (18–55 years of age) and the actual body weight for children (over 5 and less than 18 years of age).

Supportive care

For seizure prophylaxis, phenytoin was administered at 5–10 mg/kg/day (upper limit of 300 mg/kg/day) in 2–3 divided doses starting from 2 days before initiation (day −9) to 48 h after completion of BU administration (day −2). G-CSF was administered on day 1 or 5 until engraftment. For patients undergoing allogeneic HSCT, GVHD prophylaxis consisted of CYA (3 mg/kg/day by continuous i.v. infusion from day −1 in related and 3–5 mg/kg/day in unrelated transplantation) and short-term methotrexate, that is, 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 in related pairs or 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3, 6 and 11 in unrelated pairs. Mesna was administered at a dose equivalent to 120% of CY on days −3 and −2. Other supportive treatments including antiemetic administration, antibiotic treatment, transfusion support, GVHD treatment and VOD treatment were given according to the standards of each hospital.

Evaluation of clinical data

The efficacy variables were myeloablation, engraftment, relapse, overall survival (OS) and disease-free survival (DFS). The safety variables were non-relapse mortality and adverse events included convulsive seizure, VOD, acute GVHD and other organ toxicities. Engraftment was defined as an absolute neutrophil count of 0.5×10^9/l for three consecutive days. Engraftment failure was defined as the failure to reach an absolute neutrophil count of 0.5×10^9/l by day 28 after transplantation. OS was measured as the time from the day of transplantation until death from any cause, and DFS as the time from the day of transplantation until disease relapse or death from any cause. Relapse, OS and DFS were calculated using the Kaplan–Meier method.[23] non-relapse mortality was defined as any death without progression of the underlying disease. Patients were monitored daily for adverse events, hematology and transplant-related complications. After discharge, patients were followed weekly for adverse events and transplant-related complications, and monitored weekly for hematologic and biochemical data through 100 days after transplantation. The appearance of VOD by day 30 was evaluated based on any two of the major criteria as established by McDonald *et al.*[24] and Jones *et al.*[25] GVHD was graded according to the consensus criteria.[26,27] Kirin Pharma Co. Ltd. provided financial support for the medical costs associated with the conditioning regimen, including i.v. BU for enrolled patients, monitored source data and entered these data in a database. Statistical analysis was performed using SAS software (version 8.02; SAS Institute, Cary, NC, USA).

PK sampling and analysis

The objective of this study was to describe the PK characteristics of i.v. BU, with parameters including BU concentrations for the first and ninth administrations and the accumulation of i.v. BU. Plasma samples were collected from all patients at designated times, in conjunction with the first and ninth doses as follows: immediately before drug infusion and at 15, 30 and 45 min after the start of infusion, at 5 min before the end of infusion and at 15, 30, 60, 120, 180 and 240 min after completion of infusion. In addition, one sample was taken immediately before the 13th infusion and 5 min before its completion. The plasma was assayed using a gas chromatographic-mass spectrometric detection method.[10]

Plasma concentrations for first and ninth dose in individual subjects were analyzed by the non-compartmental method using WinNonlin (version 3.3; Pharsight Corp., Mountain View, CA, USA). The maximum plasma concentration (C_{max}) and the time to reach maximum plasma drug concentration (t_{max}) were observed values. The terminal half-life ($t_{1/2}$) was calculated as $\ln 2/k_{el}$, where k_{el} was the elimination rate constant, determined by log-linear regression of the terminal phase data points. The area under the plasma concentration–time curve from time 0 to infinity (AUC_{inf}) for the first dose was calculated as $AUC_{0-t} + C_t/k_{el}$, where AUC_{0-t} was the AUC from time 0 to the last detectable time, calculated using linear trapezoidal rule, and C_t was the plasma concentration at

the last detectable time. AUC at steady state (AUC_{ss}) for the ninth dose was calculated by the linear trapezoidal rule. Clearance (CL) was calculated as dose/AUC. Volume of distribution (V_z) was calculated as CL/k_{el}. CL and V_z were normalized to actual individual body weight (CL/ABW and V_z/ABW) on the day of dosing. Summary statistics were obtained for C_{max}, t_{max}, $t_{1/2}$, AUC, CL/ABW and V_z/ABW at the first and ninth dose. The AUC at dose 1 (AUC_{inf}) and dose 9 (AUC_{ss}) and the trough concentration ($C_{p.trough}$) and peak concentration ($C_{p.peak}$) at doses 9 and 13 were calculated and compared by preparing each plot.

Results

Patient characteristics

Thirty Japanese patients were registered in this prospective trial between July 2002 and October 2003. The disease characteristics and status at transplantation are given in Table 1. The median age of the patients was 30 years (range, 7–53 years). The median body mass index (BMI) was 22.65 (14.4–29.1), and the mean BMI was 22.32 ± 3.47. There were no patients with moderate or severe obesity (BMI <30). The diseases were AML in 13 patients (43%), ALL or CML in chronic phase in five patients each (17%), non-Hodgkin lymphoma (NHL) in four patients (13%) and MDS in three patients (10%). In total, 11 of the 12 patients with AML were in CR. Four of the five patients with ALL were in CR. Three patients with MDS included refractory anemia, refractory anemia with excess blasts and refractory anemia with excess blasts in transformation. Four patients with NHL included diffuse large B-cell lymphoma in CR ($n=2$), primary refractory peripheral T-cell lymphoma ($n=1$) or suspected extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue in CR ($n=1$). One patient with AML who was in remission at registration was subsequently withdrawn from protocol treatment due to onset of cardiac myopathy on day −3, and CY was changed to fludarabine. Owing to an additional protocol violation, this patient was excluded from the objective group in the analysis.

Table 1 Patient characteristics

Variables	n (%)	
	Allogeneic HSCT (n=28)	*Autologous HSCT (n=2)*
Patient age (years) (range, median)	7–53, 30	48–50, 49
5–17	3 (11)	0
18–49	20 (71)	1 (50)
50–55	5 (18)	1 (50)
Gender		
Men	18 (64)	2 (100)
Women	10 (36)	0
Disease		
AML	12 (43)	1 (50)
ALL	5 (18)	0
CML	5 (18)	0
Myelodysplastic syndrome	3 (11)	0
Non-Hodgkin lymphoma	3 (11)	1 (50)
Disease status		
CR, CP, RA	23 (82)	2 (100)
NR, RAEB, RAEB-t	5 (18)	0
Prior chemotherapy	26 (93)	2 (100)
Prior radiotherapy	2 (7)	0
Source of stem cells		
BM	18 (64)	0
Peripheral blood cells	10 (36)	2 (100)
Related or unrelated donor		
Related	19 (68)	NA
Unrelated	9 (32)	NA
Cell dose infused		
Nucleated ($\times 10^8$/kg, median, range)	2.6 (0.7–4.4)	NA
CD34 positive ($\times 10^6$/kg, median, range)	2.7 (2.1–6.3)	2.9 (2.7–3.1)

Abbreviations: CP = chronic phase; HSCT = hematopoietic SCT; NA = not applicable; NR = non-remission; RA = refractory anemia; RAEB = refractory anemia with excess of blasts; RAEB-t = refractory anemia with excess of blasts in transformation.

Engraftment

Twenty-eight patients (97%) achieved engraftment at a median of 14 days (range, 9–20 days) and 11 days after allogeneic and autologous HSCT, respectively (Table 2). One patient who received unrelated BMT for CML had graft failure. No secondary engraftment failure was observed.

Toxicity and complications

All adverse events were those that are commonly observed in HSCT and no characteristic events related to i.v. BU were observed. None of the patients had to interrupt i.v. BU treatment because of adverse events. The number of observed adverse events was 714 in 27 patients who received allogeneic HSCT and 19 in two patients who received autologous HSCT. The most frequent adverse events in the 27 allogeneic HSCT patients were vomiting and nausea in 20 patients each (74%), anorexia in 19 patients (70%), stomatitis and diarrhea in 18 patients each (67%) and headache in 17 patients (63%; Table 2). Both of the autologous HSCT patients showed stomatitis, vomiting, catheter-related infection, anorexia and dysgeusia. No seizures were observed, and with regard to other neuropsychological profiles, seven patients experienced mild dysgeusia, one moderate systemic burning sensation, one severe tremor, one severe mood change and one severe insomnia in an allogeneic setting. With regard to cardiovascular profiles, one patient experienced mild cardiac failure and the other developed moderate cardiomyopathy due to CY in the allogeneic setting, as described above. This patient had completed i.v. BU administration for 4 days and CY once. When the patient complained of chest discomfort, the heart rate was 101 beats/min, and her electrocardiography showed ST depressions in leads II, III, aVF and V_1–V_6 1 h after the completion of the first dose of CY, which made suspected diagnosis of CY-induced cardiomyopathy. The signs and symptoms subsided shortly, and the second dose of CY on day −2

Table 2 Regimen-related toxicity, engraftment, GVHD and death

Outcome	*Allogeneic HSCT (n = 28) (%)*	*Autologous HSCT (n = 2) (%)*
Toxicity		
Vomiting	21 (75)	2 (100)
Nausea	21 (75)	1 (50)
Anorexia	19 (68)	2 (100)
Stomatitis	18 (64)	2 (100)
Diarrhea	18 (64)	0 (0)
Headache	18 (64)	0 (0)
Seizure	0 (0)	0 (0)
VOD	1 (4)	0 (0)
	Allogeneic HSCT (n = 27) (%)	*Autologous HSCT (n = 2) (%)*
Engraftment	26 (96)	2 (100)
Median (days)	14	11
Range (days)	9–20	11
Graft failure	1 (4)	0 (0)
Acute GVHD	13 (48)	—
Grade I	4 (15)	—
Grade II	5 (19)	—
Grade III	2 (7)	—
Grade IV	2 (7)	—
Chronic GVHD	16 (59)	—
Death	8 (30)	0 (0)
Relapse	4 (15)	0 (0)
Non-relapse	4 (15)	0 (0)

Abbreviations: HSCT = hematopoietic SCT; VOD = venoocclusive disease.

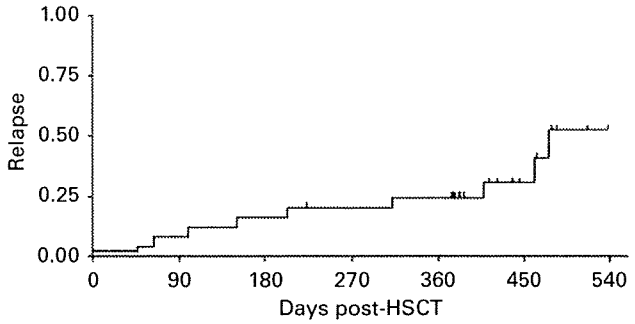

Figure 1 Disease relapse after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia and lymphoma.

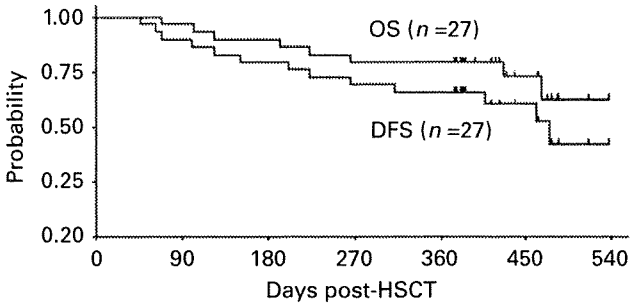

Figure 2 Overall survival and disease-free survival after i.v. BU and CY prior to allogeneic hematopoietic SCT in patients with leukemia, myelodysplastic syndrome and lymphoma.

was substituted by fludarabine with no subsequent complications.

One patient who received allogeneic HSCT was diagnosed with mild VOD on day 1 based on two diagnostic criteria,[24,25] which resolved on day 3. In another patient, elevated total bilirubin and body weight gain were found on days 60–69, and this was not confirmed to be VOD based on these criteria. Opportunistic infection occurred in 16 of 27 patients (59%), with a median onset of day 113 (range, 7–399). Pulmonary complications occurred in 7 of 27 patients (26%), with a median onset of day 149 (range, 65–335).

GVHD

Acute GVHD occurred in 13 of the 27 patients (48%) who received allogeneic HSCT; four (15%) had grade I, five (19%) grade II and two each (7%) grades III or IV (Table 2). Acute GVHD was documented in 7 of the 19 patients (37%) who received related transplantation (six had grades II–IV), and in six of the eight patients (75%) who received unrelated transplantation (three patients had grades II–IV). Acute GVHD occurred with a median onset of day 45 (range, 7–98). Chronic GVHD occurred in 16 of 27 patients (59%) with a median onset of day 133 (range, 39–239).

Causes of death

Four patients (15%) died of non-relapse causes (Table 2). One patient who received allogeneic HSCT died of multi-organ failure due to aggravated GVHD on day 69. Three patients who received allogeneic HSCT died of chronic GVHD on day 223, hepatic failure due to unknown reasons on day 266 (with extensive chronic GVHD and methicillin-resistant *staphylococcus aureus* (MRSA) pneumonia) and pneumonia due to adenovirus and cytomegalovirus on day 124. Four patients (15%) died of relapse.

Relapse and survival

Relapse occurred in 9 of the 23 evaluable allogeneic HSCT patients with leukemia and lymphoma (39%). None of the 23 evaluable patients had central nervous system relapse. The relapse rates at days 100 and 365 were 18% (95% confidence interval (CI), 0–38%) and 26% (95% CI, 8–45%), respectively (Figure 1). The median day of relapse was day 202 (range, 46–476).

OS at days 100 and 365 in allogeneic HSCT was 96% (95% CI, 88–100%) and 78% (95% CI, 62–94%), respectively, with the median follow-up of 413 days (range, 69–537 days) (Figure 2). The median day of death in eight allogeneic HSCT patients was day 208 (range, 69–467). DFS at days 100 and 365 in allogeneic HSCT was 81% (95% CI, 63–99%) and 63% (95% CI, 45–81%), respectively (Figure 2). The two autologous HSCT patients were alive disease-free at day 365.

PK analysis

Intensive PK sampling was assessed at doses 1 and 9 of i.v. BU, and peak and trough levels were obtained at dose 13. Although these analyses were completed in all 30 patients,

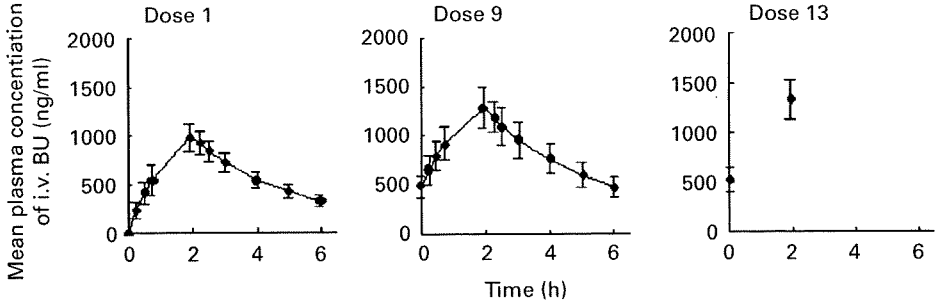

Figure 3 Pharmacokinetic results of i.v. BU at doses 1, 9 and 13 ($n = 30$).

data from one patient were excluded from the objective analysis group as noted above. All PK parameters for dose 1 were obtained from 29 patients. For dose 9, all PK parameters except for C_{max} and t_{max} were obtained from 28 patients because the last sample for one patient was collected after initiation of the next dose (Figure 3). The documented plasma concentration of i.v. BU increased over the 2-h period of infusion, with C_{max} observed in the last 5 min, and this was followed by a rapid decrease. The profile of trough and peak levels was essentially the same between doses 9 and 13.

The resulting parameters are listed in Table 3. The mean AUC for doses 1 and 9 was 1171 μmol min/l (coefficient of variation (CV) = 19%) and 1242 μmol min/l (CV = 17%), and the mean C_{max} was 994 ng/ml (CV = 12%) and 1311 ng/ml (CV = 15%), respectively. The mean CL/ABW was 2.66 ml/min/kg (CV = 17%) and 2.46 ml/min/kg (CV = 15%), respectively. V_z/ABW was 0.601/kg (CV = 9%) and 0.601/kg (CV = 11%), respectively. The AUC of the initial dose was below 1500 μmol min/l in 27 patients (90%), and this was within the range of 900–1350 μmol min/l in 21 of the 29 patients (72%).

The AUC for doses 1 and 9 are compared in Figure 4, which supports both intra- and interpatient predictability and consistency. In the patient who developed VOD, the AUC for doses 1 and 9 was 1102 and 1181 μmol min/l, respectively, whereas for the remaining patients without VOD, it was 1173 μmol min/l (CV = 19%) and 1244 μmol min/l (CV = 17%).

Pediatric patients

A 7-year-old girl with AML in first remission received allo-BMT from a matched unrelated donor. Her body weight and BMI were 17.8 kg and 14.4, respectively. Her AUC was 963.9 μmol min/l. Her regimen-related toxicities were grade 3 vomiting and grade 2 acute hemorrhagic gastritis and hypoalbuminemia. She is alive without graft failure or relapse.

A 13-year-old boy with CML in first chronic phase received allo-BMT from a matched unrelated donor. His body weight and BMI were 46.7 kg and 18.8, respectively. His AUC was 932.6 μmol min/l. His regimen-related toxicities were grade 4 anorexia and grade 2 fatigue and vomiting. He did not achieve engraftment by day 28, and he soon received a second allo-BMT from a mismatched

Table 3 Pharmacokinetics of i.v. BU ($n = 30$[a])

	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC (μmol min/l)	CL/ABW (ml/min/kg)	V_z/ABW (l/kg)
Dose 1					
Mean	999	2.64	1171	2.67	0.596
Median	997	2.66	1144	2.65	0.596
s.d.	124	0.41	216	0.44	0.054
Maximum	1320	3.52	1698	3.72	0.716
Minimum	796	1.97	811	1.94	0.483
Dose 9					
Mean	1317	2.86	1247	2.46	0.601
Median	1315	2.82	1198	2.36	0.605
s.d.	192	0.37	205	0.36	0.068
Maximum	1720	3.59	1686	3.05	0.786
Minimum	964	2.27	889	1.80	0.466

Abbreviations: ABW = actual body weight; AUC = area under the plasma concentration–time curve; CL = clearance; C_{max} = maximum plasma concentration; s.d. = standard deviation; $t_{1/2}$ = terminal half-life; t_{max} = time to observed maximum plasma concentration from dosing; V_z = volume of distribution.

[a]For dose 9, all PK parameters except for C_{max} and t_{max} were obtained from 29 patients because the last sample for one patient was collected after initiation of the next dose.

For dose 1, AUC_{inf} is shown; for dose 9, AUC_{ss} for the 6-h dosing interval is presented.

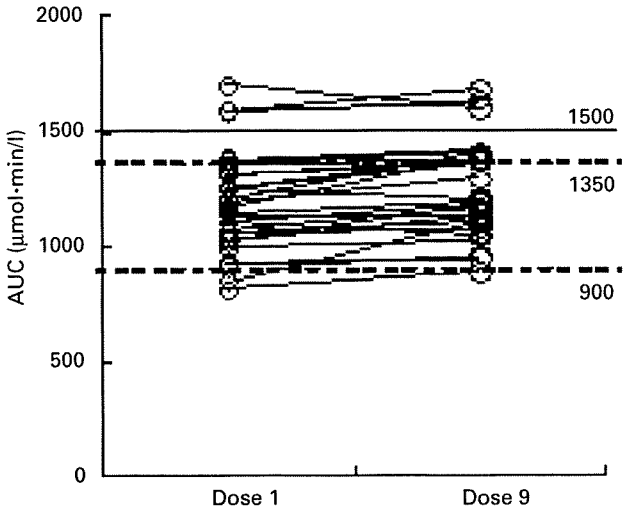

Figure 4 Individual patient area under the plasma concentration–time curve (AUC) values of i.v. BU at doses 1 and 9 ($n = 29$).

related donor. He is alive without graft failure or relapse after the second transplant.

A 17-year-old woman with AML in first relapse received allo-BMT from a matched unrelated donor. Her body weight and BMI were 43.2 kg and 17.3, respectively. Her AUC was 902.7 μmol min/l. Her regimen-related toxicities were grade 4 thrombocytopenia, grade 3 febrile neutropenia and grade 2 nausea, vomiting and stomatitis. She died of disease progression on day 193.

Discussion

It has been reported that a high steady-state concentration of BU causes toxicities including VOD,[5–10] whereas a low steady-state concentration leads to graft rejection[10–15] or relapse/progression of the disease.[11] Targeted dose adjustment of BU to maintain the overall systemic exposure within a proper range may reduce these risks.[4–7,14,15] Although it has been reported that there are ethnic differences in PK for a wide range of drugs,[28] this has not been seriously examined with i.v. BU. Therefore, we conducted this drug bioavailability study in a Japanese population. The data obtained were compared with those published mostly overseas. In this study, all observed treatment-related toxicities were as expected, with a low incidence of severe complications. One patient was clinically diagnosed with VOD. This patient showed body weight gain, liver enlargement and right upper abdominal pain, but had no jaundice. As his body weight returned to the baseline within 2 days, this could have been due to overhydration. One patient who developed graft failure had CML and underwent unrelated BMT following interferon therapy, all of which are well-known risks of graft failure.[10,29] The incidence of relapse and the survival rate in this study were similar to those in previous studies.[11,19]

In studies with an oral preparation of BU, it was unclear whether plasma levels of BU correlate with severe regimen-related toxicities.[4,6–8,11] In the pivotal study for US approval of i.v. BU, plasma levels of BU exceeded 1500 μmol min/l in two of the five patients who developed VOD,[19] whereas in our study there was no case of VOD in three patients who had a level over 1500 μmol min/l. This may suggest an ethnic difference in the PK of BU. On the other hand, a population pharmacokinetic analysis of i.v. BU is rare.[30] Our earlier small-scale study revealed high inter- and intrapatient consistency for i.v. BU pharmacokinetics.[22] However, the value of therapeutic drug monitoring remains crucial. Our study demonstrated no essential difference in PK analysis from earlier published Western data,[19] and this supports the notion that racial factors may not seriously influence the bioactivity of i.v. BU.

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HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism

Takakazu Kawase,[1] Keitaro Matsuo,[1] Koichi Kashiwase,[2] Hidetoshi Inoko,[3] Hiroh Saji,[4] Seishi Ogawa,[5] Shunichi Kato,[6] Takehiko Sasazuki,[7] Yoshihisa Kodera,[8] and Yasuo Morishima,[9] for The Japan Marrow Donor Program

[1]Division of Epidemiology and Prevention, Aichi Cancer Center, Nagoya; [2]Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo; [3]Division of Molecular Science, Tokai University School of Medicine, Isehara; [4]HLA Laboratory, NPO, Kyoto; [5]The 21st Century COE Program, Graduate School of Medicine, University of Tokyo, Tokyo; [6]Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara; [7]International Medical Center of Japan, Tokyo; [8]Japanese Red Cross Nagoya First Hospital, Nagoya; and [9]Department of Hematology and Cell Therapy, Aichi Cancer Center, Nagoya, Japan

The finding that the risk of relapse in hematologic malignancy decreases after allogeneic hematopoietic stem cell transplantation (HSCT) has lead to the concept of a graft-versus-leukemia (GVL) effect. However, this beneficial effect is considered to be frequently offset by graft-versus-host disease (GVHD). Thus, improving HSCT outcomes by separating GVL from GVHD is a key clinical issue. This cohort study registered 4643 patients with hematologic malignancies who received transplants from unrelated donors. Six major human leukocyte antigen (HLA) loci were retrospectively genotyped. We identified 4 HLA-Cw and 6 HLA-DPB1 mismatch combinations responsible for a decreased risk of relapse; of these, 8 of 10 combinations were different from those responsible for severe acute GVHD, including all 6 of the HLA-DPB1 combinations. Pairs with these combinations of HLA-DPB1 were associated with a significantly better overall survival than were completely matched pairs. Moreover, several amino acid substitutions on specific positions responsible for a decreased risk of relapse were identified in HLA-Cw, but not in HLA-DPB1. These findings might be crucial to elucidating the mechanism of the decreased risk of relapse on the basis of HLA molecule. Donor selection made in consideration of these results might allow the separation of GVL from acute GVHD, especially in HLA-DPB1 mismatch combinations. (Blood. 2009;113:2851-2858)

Introduction

The use of allogeneic hematopoietic stem cell transplantation (HSCT), an established treatment for hematologic malignancies, is associated with several immunologic events with contrary effects in the recipient. In graft-versus-host disease (GVHD), for example, graft immune cells attack host organs, whereas in the graft-versus-leukemia (GVL) effect, they eradicate residual leukemia cells.[1-3] GVL is likely to function not only in hematologic malignancies but also in solid tumors, particularly breast cancer and renal cell carcinoma,[4-6] in which it is referred to as the graft-versus-tumor (GVT) effect. Because both GVL and GVHD are caused by either or both major and minor histocompatibility antigen mismatches between donor and recipient, the beneficial effect of allogeneic HSCT due to GVL is thought to be frequently offset by GVHD. Thus, improving HSCT outcome by separating GVL from GVHD is a key clinical issue. Importantly, however, while most such efforts have been in the area of minor histocompatibility antigen,[7] few researchers have approached this problem in terms of the major histocompatibility antigen.

We recently identified 16 human leukocyte antigen (HLA) mismatch combinations associated with a high risk of severe acute GVHD. Results showed that the overall number of these high-risk mismatches was strongly associated with the occurrence of severe acute GVHD and poor overall survival (OS).[8] We speculated that the intensity of GVL and acute GVHD in any particular mismatch might not necessarily be parallel, and that among HLA mismatch combinations not inducing severe acute GVHD, those that induce strong GVL might occur. In other words, the hypotheses of this study were that particular mismatch combinations allow the separation of GVL from acute GVHD and that specific amino acid substitutions in HLA molecules contribute to this mechanism.

As part of efforts to improve donor selection and allogeneic HSCT outcomes, we identified HLA mismatch combinations that resulted in a decreased risk of relapse in all 6 major HLA loci and compared them with mismatch combinations carrying a high risk of severe acute GVHD. Further, we investigated specific amino acid substitution positions in the HLA molecule responsible for a decreased risk of relapse.

Methods

Patients

This study was conducted using clinical data that were collected prospectively at transplant centers participating in the Japan Marrow Donor Program. Patients who received a first transplant of T cell–replete marrow for a hematologic malignancy from a serologically HLA-A, -B, and -DR antigen-matched unrelated donor between January 1993 and December 2005 through the Japan Marrow Donor Program (n = 4643) were registered. Eligible diagnoses included acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML), which included only de novo AML;

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Table 1. Patient characteristics

		A locus		B locus		C locus		DRB1 locus		DQB1 locus		DPB1 locus	
	Total	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch
	4643	4018	625	4351	292	3308	1335	3718	925	3597	1046	1584	3059
Median age, y	31.5	31.8	29.6	31.7	28.3	31.8	30.9	31.7	30.9	31.7	30.8	31.8	31.4
Sex, donor/patient													
Male/male	1904	1673	231	1769	135	1387	517	1551	353	1492	412	678	1226
Male/female	923	789	134	874	49	650	273	734	189	704	219	299	624
Female/male	894	747	147	843	51	634	260	693	201	672	222	268	626
Female/female	922	809	113	865	57	637	285	740	182	729	193	339	583
Disease													
ALL	1464	1267	197	1372	92	1051	413	161	303	1132	332	452	1012
AML	1571	1360	211	1478	93	1114	457	1255	316	1224	347	574	997
CML	979	827	152	905	74	682	297	779	200	746	233	343	636
ML	564	507	57	536	28	43	146	468	96	49	118	192	372
MM	65	57	8	60	5	418	22	55	10	446	16	23	42
Risk of leukemia relapse*													
Standard risk	1684	1485	199	1588	96	1184	500	1375	309	1322	362	572	1112
High risk	1909	1607	302	1772	137	1365	544	1485	424	1451	458	642	1267
Disease other than leukemia	1050	926	124	991	59	759	291	858	192	824	226	370	680
GVHD prophylaxis													
Cyclosporine-based	2503	2159	344	2346	157	1802	701	2107	396	2030	473	881	1622
Tacrolimus-based	2140	1859	281	2005	135	1506	634	1611	529	1567	573	703	1437
ATG													
ATG	152	112	40	135	17	102	50	110	42	118	34	51	101
Non-ATG	4491	3906	585	4216	275	3206	1285	3608	883	3479	1012	1533	2958
Preconditioning													
TBI regimen	3687	3175	512	3445	242	2623	1064	2933	754	2834	853	1242	2445
Non-TBI regimen	956	843	113	906	50	685	271	785	171	763	193	342	614

ATG indicates antithymocyte globulin; and TBI, total body irradiation.

*Standard risk for leukemia relapse was defined as the status of the first complete remission of AML and ALL and the first chronic phase of CML at transplant, while high risk was defined as a more advanced status than standard risk in AML, ALL, and CML. Disease other than leukemia was defined as other than ALL, AML, and CML.

chronic myeloid leukemia (CML); malignant lymphoma (ML); and multiple myeloma (MM).

Patient characteristics are shown in Table 1. A final clinical survey of the patients was completed by December 2006. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval for the study was obtained from the Institutional Review Board of Aichi Cancer Center and the Japan Marrow Donor Program.

HLA typing of patients and donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were identified by previously described methods in all 4643 pairs at the Japanese Red Cross Tokyo Metropolitan Blood Center.[8,9]

Matching of HLA allele between patient and donor

HLA allele mismatch among the donor-recipient pair was scored when the recipient's alleles were not shared by the donor (graft-versus-host vector) for all analyses.

Definition of relapse

Relapse was defined as the recurrence of malignancy as detected by the parameter by which the malignancy was first detected, namely marrow morphology; flow cytometry; cytogenetic studies, including fluorescence in situ hybridization; electrophoresis; immunofixation assays; polymerase chain reaction-based assays for disease markers; or imaging results. The day of relapse was defined as the day on which the respective clinical, hematologic, cytogenetic, or molecular relapse was recognized.

Definition of amino acid substitution

Amino acid sequences of HLA-Cw and -DPB1 molecules were obtained from the IMGT/HLA sequence database.[10] For example, Tyr99C-Phe99C indicated an amino acid substitution at position 99 in the HLA-C molecule in which the donor had tyrosine and the patient had phenylalanine. Substituted amino acids in HLA-Cw and -DPB1 are summarized in Tables S1 and S2 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Statistical analysis

OS rate was assessed using the Kaplan-Meier product limit method. To eliminate the effect of competing risk, the cumulative incidence of relapse was assessed using a previously described method.[11,12] The competing event for relapse was defined as death without relapse. Impact by the factor of interest was assessed using the log rank test. The impact of HLA allele mismatch combinations and the position and type of amino acid substitution (for example, alanine, arginine, and asparagine) in HLA molecules were evaluated using multivariable Cox regression analysis[13] for OS and the occurrence of acute GVHD, while the risk of relapse was evaluated using the multivariable proportional hazard modeling of subdistribution functions in competing risks.[14]

HLA mismatch combinations were evaluated for each locus separately. When the locus of interest was evaluated, we allowed the other loci to be mismatched, with the status of such mismatches adjusted for in the same way as other confounders. The HLA match and HLA one-allele mismatched in every locus were analyzed. For example, the A*0206-A*0201 mismatch combination meant that the donor had HLA-A*0206, the recipient had HLA-A*0201, while another HLA-A allele of the donor and recipient was identical. This mismatch was compared with the HLA-A allele match. Mismatch combinations that had 9 or fewer pairs were combined together as "other mismatch." The model was constructed with mismatch combinations, mismatch status in other loci (match, 1 allele mismatched, and 2 alleles mismatched, as an ordinal variable), and potential confounders. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), transplant year, type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (cyclosporine [CSP] vs tacrolimus [FK]), ATG (vs no ATG), and

preconditioning (TBI vs non-TBI). These confounders were used in all analyses to maintain the comparability of results.

The impact of position and type of amino acid substitutions in HLA molecules was evaluated in pairs with oneallele mismatched in HLA-Cw and -DPB1 separately. The amino acid positions we analyzed were all positions at which an amino acid was substituted in the respective locus. We analyzed the impact of each amino acid substitution on each position separately. Multivariable models were constructed to include the position and type of amino acid substitution, mismatch status in other loci (match, 1 allele mismatched, and 2 alleles mismatched as an ordinal variable) and the confounders described above. A P value less than .05 was considered statistically significant. All statistical tests were 2-sided. All analyses were performed using STATA version 10.0 (StataCorp, College Station, TX) and R version 2.5.1 (The R Foundation for Statistical Computing, www.r-project.org).

Validation of statistical analysis

Statistical analyses were validated using the bootstrap resampling method.[15] Briefly, we estimated the measure of association with resampled data drawn repeatedly from the original data. Although approximately 100 to 200 bootstrapped samples are generally sufficient,[16] we used 1 000 bootstrap samples for all analysis validations. Further, we judged the results of analysis as statistically significant only when the results of both base analysis and analysis validation using bootstrap resampling were significant; cases in which the result of base analysis was significant but that of analysis validation using bootstrap resampling was not are indicated by an asterisk next to the P value of the base analysis.

Results

Impact of HLA allele mismatches in locus level on relapse

The number of mismatched alleles of HLA-Cw (1 allele mismatched: hazard ratio [HR], 0.68; 95% confidence interval [CI], 0.58-0.80; 2 alleles mismatched: HR, 0.43;95% CI, 0.24-0.75) and HLA-DPB1 (1 allele mismatched: HR, 0.80; 95% CI, 0.70-0.92; 2 alleles mismatched: HR, 0.62; 95% CI, 0.51-0.75) was strongly associated with a decreased risk of relapse. In contrast, no associations were seen for HLA-A (1 allele mismatched: HR, 1.00; 95% CI: 0.82-1.22; 2 alleles mismatched: HR, 0.79; 95% CI, 0.28-2.28), HLA-B (1 allele mismatched: HR, 1.06; 95% CI, 0.79-1.41; 2 alleles mismatched: not applicable), HLA-DRB1 (1 allele mismatched: HR, 0.93; 95% CI, 0.74-1.18; 2 alleles mismatched: HR, 1.18, 95% CI: 0.53-2.63) or HLA-DQB1 (1 allele mismatched: HR, 1.12; 95% CI, 0.90-1.40; 2 alleles mismatched: HR, 0.73; 95% CI, 0.35-1.52; Figure 1; Table 2).

Impact of HLA mismatch combinations on relapse

Four mismatch combinations in HLA-Cw and 6 in HLA-DPB1 were significantly associated with a decreased risk of relapse (Tables 3 and S3). In contrast, mismatch combinations in HLA-A, -B, -DRB1, and -DQB1 were not significantly associated with differences in risk of relapse (data not shown). The 10 HLA mismatch combinations associated with lower risks of relapse were Cw*0102-Cw*1402 (HR not estimated due to no event), Cw*0801-Cw*0102 (HR not estimated), Cw*1402-Cw*0304 (HR not estimated), Cw*1502-Cw*1402 (HR, 0.28; 95% CI, 0.09-0.88), DPB1*0402-DPB1*0201 (HR, 0.32, 95% CI, 0.12-0.87), DPB1*0501-DPB1*0201 (HR, 0.67; 95% CI:0.50-0.91), DPB1*0501-DPB1*0401 (HR, 0.36; 95% CI, 0.13-0.98), DPB1*0501-DPB1*0402 (HR, 0.55; 95% CI, 0.33-0.93), DPB1*0901-DPB1*0201 (HR, 0.37; 95% CI, 0.14-0.96), and DPB1*1301-DPB1*0201 (HR not estimated; Tables 3 and S3). All 10 HLA mismatch combinations were also significant on validation analysis using the bootstrap resampling method. We speculated that these mismatch combinations would mainly decrease the risk of relapse due to GVL, so we tentatively call them GVL mismatch combinations.

Evaluation of clinical importance of GVL mismatch combinations

We evaluated the clinical importance of GVL mismatch combinations in HLA-Cw and -DPB1. All analyses in this section were conducted in matched pairs other than the evaluated locus. In HLA-C mismatch, the small number of patients with GVL mismatch combinations (n = 13) in matched pairs at the allele level for HLA-A, -B, -DRB1, -DQB1, and -DPB1 prevented comprehensive analysis. We evaluated the GVL mismatch combinations of HLA-DPB1 in matched pairs for HLA-A, -B, -Cw, -DRB1, and -DQB1. Pairs with HLA-DPB1 mismatch were divided into 2 groups, those with a GVL mismatch combination and those with mismatch combinations other than GVL mismatch combinations. These were then compared with 12/12 matched pairs for association with severe acute GVHD, relapse, and OS (Table 4). The curve of the cumulative incidence of OS is shown in Figure 2. Multivariable analysis revealed that although OS was similar between the 12/12 matched pairs and the pairs with mismatch combinations other than GVL mismatch combinations, it was significantly improved in pairs with a GVL mismatch combination (Table 4). In terms of mortality due to relapse according to HLA-DPB1 matching status and whether the mismatch combinations were GVL mismatch combinations, the HLA-DPB1 matched group, HLA-DPB1 1 allele mismatched group, and GVL mismatch combination group showed an expected decreased mortality due to relapse (20.0%, 15.3%, and 10.5%, respectively). Further, mortality due to relapse in the GVL mismatch combination group was significantly lower than that in the HLA-DPB1 1 allele mismatched group (P = .049). We conducted the same analyses with stratification by leukemia type (ALL, AML, or CML) and found that the myeloid malignancies (AML and CML) had the same tendency (Table 4). In particular, in CML, GVL mismatch combinations in HLA-DPB1 were associated with a significantly reduced risk of relapse (HR, 0.14; 95% CI, 0.03-0.55) and significantly improved OS relapse (HR, 0.50; 95% CI, 0.25-0.98).

Impact of position and type of amino acid substitutions of HLA molecules on relapse

We surveyed all substituted positions in HLA-Cw and -DPB1 and found 159 specific amino acid substitutions at 55 positions in HLA-Cw and 55 specific amino acid substitutions at 19 positions in HLA-DPB1 (Tables S1,S2). Analysis revealed 3 specific amino acid substitutions responsible for a decreased risk of relapse in HLA-C, namely Ser9C-Tyr9C (HR, 0.53; 95% CI, 0.30-0.92), Phe99C-Tyr99C (HR, 0.52, 95% CI, 0.30-0.91), and Arg156C-Leu156C (HR, 0.59; 95% CI, 0.37-0.92). In contrast, no decrease in the risk of relapse was seen for substitutions in HLA-DPB1 (Table 5). However, Tyr9C-Ser9C and Tyr99C-Phe99C were strongly linked (see "Discussion"). These specific amino acid substitutions were all significant on validation analysis using the bootstrap resampling method.

Discussion

Improving outcomes in allogeneic HSCT for hematologic malignancies by separating GVL from GVHD is considered a key clinical

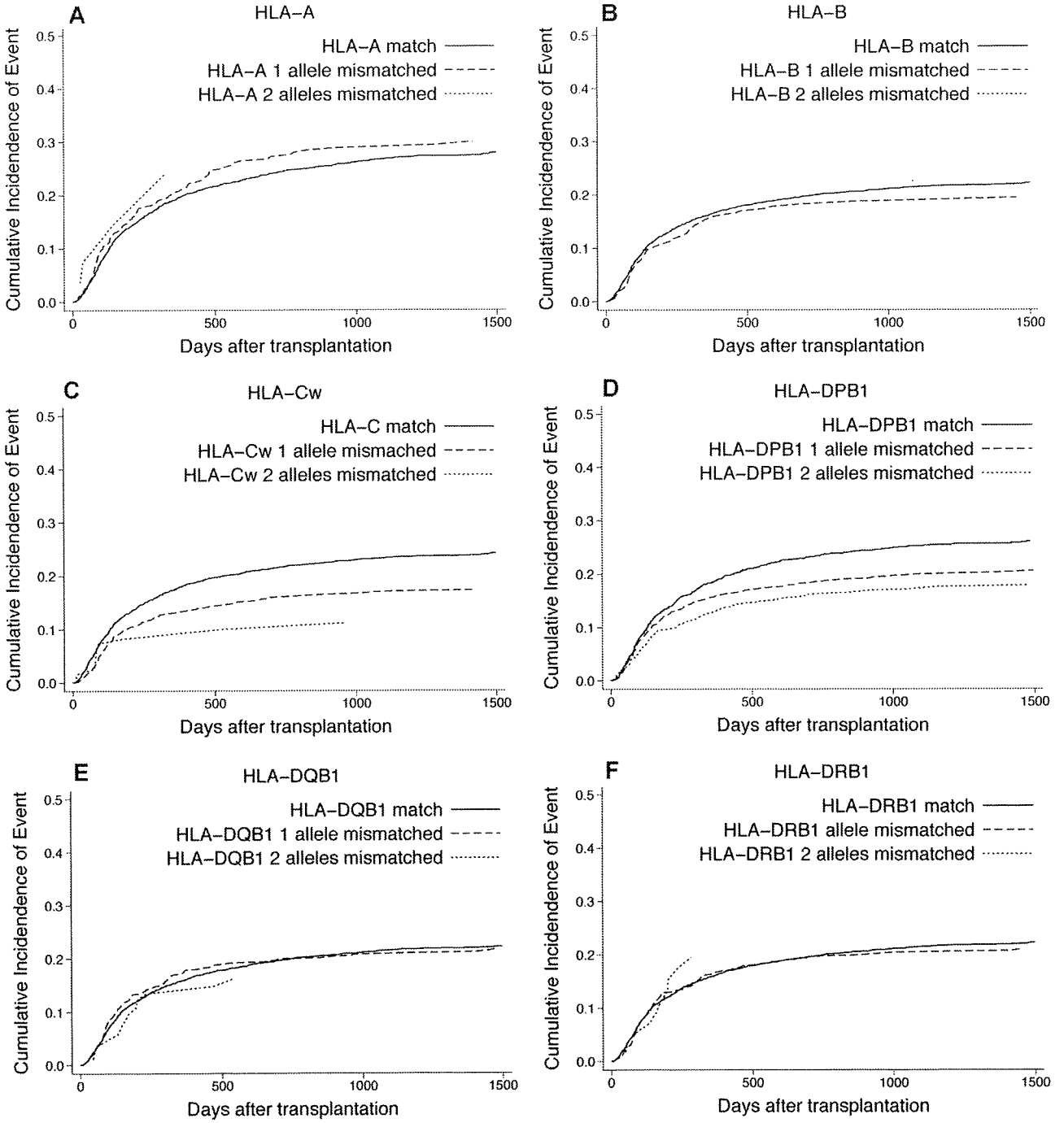

Figure 1. Impact of individual HLA locus mismatches on relapse. Cumulative incidence of relapse for each HLA locus. [—] indicates matched pairs in each locus; [···], 1-allele mismatched pairs in each locus; and [...], 2-allele mismatched pairs in each locus.

challenge. Here, our analysis demonstrated that several donor-recipient HLA mismatch combinations and specific amino acid substitutions in HLA molecules were associated with a decreased risk of relapse, and, in some cases, no significant increase in the risk of severe acute GVHD. These findings suggest that GVL might be separated from severe acute GVHD by selection of suitable HLA mismatch combinations.

We recently reported 16 significant high-risk HLA allele mismatch combinations for severe acute GVHD in 6 HLA loci, a number of which were highly associated with the occurrence of severe acute GVHD and worse OS.[8] Of note, a group of pairs with mismatches other than severe acute GVHD high-risk mismatches showed an incidence of severe acute GVHD and OS rates almost equal to those of 12/12 matched pairs. In the present study, we elucidated a total of 10 mismatch combinations that were significantly associated with a decreased risk of relapse, which we termed GVL mismatch combinations. Of course, it is possible that some mismatch combinations not classified as GVL mismatch combinations might actually induce strong GVL. Misclassification might have occurred as a result of insufficient statistical power due to the relatively small number of patients in the subcategories. Among these mismatch combinations, 2 of 4 in HLA-Cw were identical to the severe acute GVHD high-risk combinations; a third had a marginal effect on the occurrence of severe acute GVHD, while the

Table 2. Impact of HLA mismatches in allele level on relapse

	All diseases		
	n	HR (95% CI)	P
HLA-A matched	4018	1.00 (ref)	
HLA-A 1 allele mismatched	597	1.00 (0.82-1.22)	.99
HLA-A 2 alleles mismatched	28	0.79 (0.28-2.28)	.67
HLA-B matched	4351	1.00 (ref)	
HLA-B 1 allele mismatched	288	1.06 (0.79-1.41)	.7
HLA-B 2 alleles mismatched*	4	ND	ND
HLA-C matched	3308	1.00 (ref)	
HLA-C 1 allele mismatched	1212	0.68 (0.58-0.80)	<.001
HLA-C 2 alleles mismatched	123	0.43 (0.24-0.75)	.003
HLA-DRB1 matched	3718	1.00 (ref)	
HLA-DRB1 1 allele mismatched	866	0.93 (0.74-1.18)	.56
HLA-DRB1 2 alleles mismatched	59	1.18 (0.53-2.63)	.68
HLA-DQB1 matched	3597	1.00 (ref)	
HLA-DQB1 1 allele mismatched	958	1.12 (0.90-1.40)	.30
HLA-DQB1 2 alleles mismatched	88	0.73 (0.35-1.52)	.40
HLA-DPB1 matched	1584	1.00 (ref)	
HLA-DPB1 1 allele mismatched	2190	0.80 (0.70-0.92)	.002
HLA-DPB1 2 alleles mismatched	869	0.62 (0.51-0.75)	<.001

Each group was compared with the matched group in each locus after adjusting for other matching status of HLA, sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (CSP vs FK), ATG vs no ATG), and preconditioning (TBI vs non-TBI).

ref indicates reference; and ND, not determined.

*Comprehensive analysis could not be performed due to the small number of cases.

fourth combination was different from acute GVHD high-risk mismatch combinations. In contrast, all 6 mismatch combinations in HLA-DPB1 were different from acute GVHD high-risk mismatch combinations (Table 3). As expected, HLA-A, -B, -Cw, -DRB1, and -DQB1 matched pairs with GVL mismatch combinations of HLA-DPB1 were associated with significantly better OS than 12/12 matched pairs (Table 4; Figure 2), indicating that the beneficial antitumor effect of GVL mismatch combinations in HLA-DPB1 would not be offset by the effect of severe acute GVHD. We speculate that conformational changes of HLA molecules in each mismatch combination control the intensity of the acute GVHD and GVL effect, as described later in "Discussion" and in our previous report[8]; namely, conformational changes of HLA molecules in GVL mismatch combinations in HLA-DPB1 induce strong GVL with mild or no acute GVHD. These findings suggest that HLA mismatch selection according to these results might improve HSCT outcomes over those obtained with a complete match. The same tendency was seen for AML and CML, whereas the effect of GVL mismatch combination in the HLA-DPB1 allele in ALL patients would be weaker than in the other leukemia types (Table 4). Comprehensive analyses for ML and MM could not be done because of the small number in each group. Thus, the effects of GVL mismatch combination vary according to disease type and may also change according to other factors, including particular cytogenetic abnormalities.

Recent research has shown that HLA-Cw and -DPB1 mismatch at the allele level is strongly associated with a decreased risk of relapse.[17,18] These findings were confirmed in the present large cohort. In addition, the present study also clarified that the mismatching of 2 alleles in either the HLA-Cw or -DPB1 locus had a stronger association with decreased risk than respective mismatching of one allele. Moreover, no association whatsoever was seen for

Table 3. GVL mismatch combinations

Mismatch combination, donor-recipient	n	HR (95% CI)	P
Cw*0102-Cw*1402*†	13	ND	ND
Cw*0801-Cw*0102*†	10	ND	ND
Cw*1402-Cw*0304†	20	ND	ND
Cw*1502-Cw*1402	43	0.28 (0.09-0.88)	.030
DPB1*0402-DPB1*0201*	54	0.32 (0.12-0.87)	.026
DPB1*0501-DPB1*0201*	301	0.67 (0.50-0.91)	.009
DPB1*0501-DPB1*0401*	48	0.36 (0.13-0.98)	.046
DPB1*0501-DPB1*0402*	112	0.55 (0.33-0.93)	.026
DPB1*0901-DPB1*0201*	43	0.37 (0.14-0.96)	.042
DPB1*1301-DPB1*0201*†	20	ND	ND

As an example of the mismatch combination analysis, the Cw*0102-Cw*1402 mismatch combination meant that the donor has HLA-Cw*0102, the recipient has HLA-Cw*1402 and another HLA-Cw allele of each donor and recipient was identical. Each mismatch pair in HLA-Cw was compared with the HLA-Cw allele match, and each mismatch pair in HLA-DPB1 was compared with the HLA-DPB1 allele match. All indicated results were concurrently significant in both the base analysis and validation analysis using bootstrap resampling.

ND indicates not determined.

*Mismatch combinations that were not significantly associated with a higher occurrence of severe acute GVHD in our previous study.[8] However, the Cw*0102-Cw*1402 mismatch combination has a marginal effect on the occurrence of severe acute GVHD; that is, Cw*0102-Cw*1402 was significantly associated with a higher occurrence of severe acute GVHD in base analysis, but not in validation analysis.

†HR was not estimated due to the lack of an event in this group.

Table 4. Clinical importance of GVL mismatch combinations in HLA-DPB1 mismatch

		Acute GVHD		Relapse		OS*	
All diseases	**n**	**HR (95% CI)**		**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
HLA-DPB1 matched	864	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	808	1.34 (1.03-1.74)	.028	0.83 (0.68-1.01)	0068	0.96 (0.83-1.12)	.62
GVL mismatch combination	258	1.18 (0.81-1.73)	.375	0.47 (0.33-0.67)	<.001	0.75 (0.59-0.94)	.012
ALL							
HLA-DPB1 matched	250	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	263	1.56 (0.96-2.54)	.067	0.85 (0.6-1.19)	.33	1.10 (0.85-1.43)	.48
GVL mismatch combination	80	1.27 (0.63-2.57)	.5	0.75 (0.45-1.26)	.28	0.95 (0.65-1.39)	.8
AML							
HLA-DPB1 matched	308	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	264	1.47 (0.9-2.39)	.13	0.83 (0.61-1.14)	.26	0.95 (0.74-1.23)	.72
GVL mismatch combination	89	1.25 (0.62-2.5)	.54	0.44 (0.24-0.78)	.006	0.71 (0.48-1.06)	.1
CML							
HLA-DPB1 matched	176	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	162	1.25 (0.74-2.14)	.41	0.69 (0.40-1.20)	.19	0.93 (0.65-1.33)	.69
GVL mismatch combination	54	1.13 (0.51-2.47)	.66	0.14 (0.03-0.55)	.005	0.50 (0.25-0.98)	.041

Each group was compared with the HLA-DPB1 matched group. Confounders considered were sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (CSP vs FK), ATG vs no ATG), and preconditioning (TBI vs non-TBI). ref indicates reference.

*The HR indicates the likelihood that OS will be shorter (if HR > 1) or longer (HR < 1) than when the HLA type matches (ie, the Ref condition).

HLA-A, -B, -DRB1, or -DQB1 (Figure 1; Table 2). Furthermore, all 10 GVL mismatch combinations were elucidated from mismatch combinations of HLA-Cw and HLA-DPB1 (Tables 3 and S3), although we also analyzed HLA-A, -B, -DRB1, and -DQB1. These findings indicate that GVL after allogeneic HSCT is mainly induced by HLA-Cw and -DPB1, not HLA-A, -B, -DRB1 or -DQB1, although the role of each HLA locus might vary with the type of disease.[18] There are 3 possible explanations for this. First, the relative expression of HLA-Cw and -DPB1 on malignant cells may be higher than that on normal hematopoietic cells; second, HLA-Cw and -DPB1 may be preferentially expressed on malignant stem cells; and third, surface expression of a few key molecules—such as major histocompatibility complex (MHC), adhesion, and costimulatory molecules—on malignant cells may determine the effect of each HLA locus on GVL.[19-21] In other words, some molecules might stimulate GVL of HLA-Cw or -DPB1, and other molecules might block GVL of other than HLA-Cw and -DPB1. Further investigation of this question is warranted.

In this study, 3 specific amino acid substitutions responsible for GVL at positions 9, 99, and 156 were identified in HLA-Cw, of which only 2, Ser9C-Tyr9C and Phe99C-Tyr99C, were strongly linked in our sample. We were therefore unable to determine which substitutions are the main contributors to the effect of interest (Table 5). These amino acid positions, 9, 99, and 156, were identical to those we elucidated in our previous study as responsible for severe acute GVHD.[8] These findings suggest that these 3 amino acid positions are important determinants of alloreactivity. Although position 156 of the HLA molecule has been shown to modify T-cell alloreactivity in vitro in HLA-A2,[22-24] -B35,[25] and -B44,[26] to our knowledge, the present study is the first to identify positions 9 and 99. On the other hand, substituted amino acids were not necessarily identical. In Ser9C-Tyr9C and Phe99C-Tyr99C substitutions, for example, the substituted amino acid position was identical with that responsible for severe acute GVHD, whereas the substituted amino acids were inverse between donor and recipient, even though both substituted position and amino acids were identical in the Arg156C-Leu156C substitution. These findings suggest that Ser9C-Tyr9C and Phe99C-Tyr99C might play an important role in separating GVL from acute GVHD in HLA-Cw mismatch, although the mechanism requires further molecular clarification.

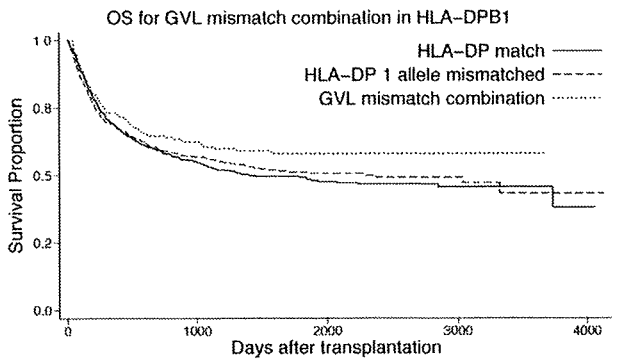

Figure 2. Clinical importance of GVL mismatch combinations in HLA-DP mismatch. Kaplan-Meier estimates of survival according to HLA-DPB1 mismatch status. The solid line indicates HLA-DPB1 matched pairs; the short broken line, HLA-DPB1 1 allele mismatched but not GVL mismatch combinations; and the dotted line, HLA-DPB1 1 allele mismatched (GVL mismatch combinations). All groups are HLA-A, -B, -C, -DRB1, and -DQB1 matched pairs.

Table 5. Impact of position and type of amino acid substitution of HLA molecules on relapse

Position and amino acid substitution in HLA-C (donor-recipient)	n	HR (95% CI)	*P*
Ser9C-Tyr9C	152	0.53 (0.30-0.92)	.024
Phe99C-Tyr99C	153	0.52 (0.30-0.91)	.022
Arg156C-Leu156C*	225	0.59 (0.37-0.92)	.020

The impact of position and type of amino acid substitution in HLA molecules was evaluated in pairs with HLA one-locus mismatch in HLA-C and -DPB1 separately. For example, Tyr9C-Ser9C indicated amino acid substitutions of position 9 in the HLA-C molecule in which the donor had tyrosine and the patient serine. The impact of position and kind of amino acid substitution in each HLA molecule was evaluated in pairs with HLA one locus mismatch in each HLA locus separately. Pairs that substituted a specific amino acid at each position were compared with amino acid matched pairs at that position.

No significant amino acid substitutions were found in HLA-DPB1.

All indicated results were concurrently significant in both base analysis and validation analysis using bootstrap resampling.

The 2 specific amino acid substitutions Tyr9C-Ser9C and Tyr99C-Phe99C were strongly linked in our sample.

*An amino acid substitution that was significantly associated with a higher occurrence of severe acute GVHD in our previous study.[8]

With regard to specific amino acid substitutions of HLA-DPB1, we found no significant association among these with a decreased risk of relapse. Shaw et al[27] reported that mismatches at position 57 and 65 in the HLA-DPB1 molecule were associated with transplant complications, but not with GVHD or relapse, which is consistent with our present data. We speculate that, compared with MHC class I, the conformational diversity of MHC class II and peptide complex hampers the identification of strict rules of association between specific amino acid substitutions in MHC class II molecules and the occurrence of alloreaction such as GVHD and GVL. In HLA class I, binding peptides are held by their ends, whereas peptides bind to HLA class II by attachment in the middle, allowing them to vary greatly in length.[28]

Given that this analysis was conducted using a Japanese cohort of patients who received transplants through the Japan Marrow Donor Program, the applicability of our data to other ethnic groups warrants discussion. We speculate that the effect of alloreaction is a reflection and summation of HLA allele mismatch combinations. Discrepancies in the effect of HLA locus on alloreactions between ethnically diverse transplantation might be explained by the proportions of each HLA mismatch combination in each HLA locus. In HLA-DPB1, on the other hand, the allele variations between white and Japanese populations are relatively close, hence our findings in HLA-DPB1 might also be useful for white populations. Regarding HLA-Cw and killer immunoglobulin-like receptor (KIR) incompatibility, we previously reported adverse effects in unrelated T cell–replete HSCT through the Japan Marrow Donor Program,[18] although Ruggieri et al[29] demonstrated that beneficial effects were shown in T-cell depleted haploidentical transplantation. We speculated that in vivo and/or in vitro T-cell depletion could account for this discrepancy.[30] Therefore, results for mismatch combinations in HLA-Cw obtained in other populations treated in other settings may differ from our results. Nevertheless, clarification of these questions would require the same study in other ethnic populations.

Given the general acceptance that GVL is more closely correlated with chronic GVHD than acute GVHD,[3] separating GVL from chronic GVHD may be more difficult than separating it from acute GVHD. On this basis, our results suggest that GVL could be separated from acute GVHD in HSCT from a specific HLA partially mismatched donor. Clarification of whether GVL can also be separated from chronic GVHD requires further study.

In conclusion, we identified 4 HLA-C and 6 HLA-DPB1 mismatch combinations that decrease the risk of relapse in patients after HSCT. Eight of 10 GVL combinations were different from those responsible for severe acute GVHD. In particular, all 6 GVL combinations in HLA-DPB1 were different. Further, pairs with these GVL combinations of HLA-DPB1 were associated with significantly better OS than completely matched pairs. These findings suggest that donor selection according to these results could separate the occurrence of GVL from acute GVHD, especially in HLA-DPB1. Further, amino acid substitutions on specific positions responsible for this decreased risk of relapse were also elucidated in HLA-C, but not in HLA-DPB1. Our finding that specific amino acid substitutions decrease the risk of relapse might be key to revealing the mechanism of the decreased risk of relapse due to GVL with regard to the HLA molecule.

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Authorship

Contribution: T.K., Y.M., T.S., S.O., and Y.K. participated in the conception of this study; K.K., H.I., and H.S. participated in the assessment of histocompatibility; Y.M. and S.K. participated in the execution of transplantation; T.K. and K.M. participated in the statistical data analysis; T.K. and Y.M. wrote the paper; and all authors checked the final version of the manuscript.

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Correspondence: Yasuo Morishima, Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, 464-8681, Japan; e-mail: ymorisim@aichi-cc.jp.

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