Table 4 Crude rate and crude relative risk of MDS in Nagasaki "Open cohort" and LSS cohort (data from reference [11])

Exposure distance from the hypocenter (km)	<1.5	1.5–2.999	>3	Total
Crude rate	43.1	17.6	12.8	15.9
Crude relative risk	3.2	1.4	Reference	
Bone marrow dose by DS02 (Gy)	≥1	0.005–0.999	<0.005	Total
Crude rate	80.7	26.6	10.5	17.4
Crude relative risk	8.1	1.4	Reference	

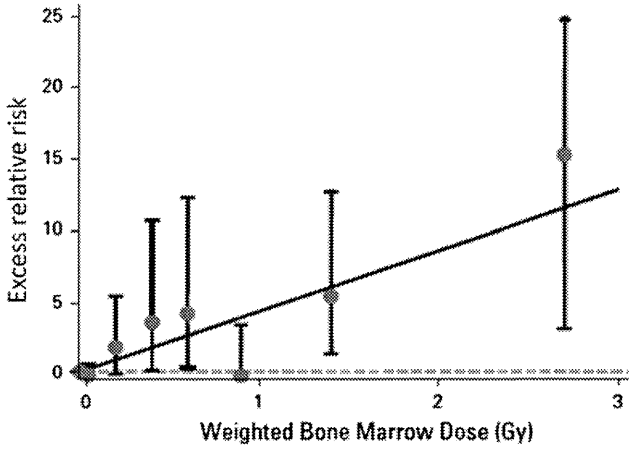

Fig. 6 Fitted relationship between excess relative risk of MDS and bone marrow dose. *Solid line* shows the relative risk of MDS by dose, and *dotted line* for control (data from reference [11])

median age at diagnosis were 71.0 years (range 42.0–96.6 years). For MDS in LSS, those were 16.5 years (range 2.5–48.8 years) and 72.4 years (range 48.5–94.3 years), respectively. Using these data, crude incidence and crude relative risk of MDS were analyzed in each category. The crude MDS incidence rates in the NOC and LSS cohort were 15.9 and 17.4 patients per 100,000 person-year, respectively (Table 4). MDS rates were higher for men than for women and increased with age at exposure as total. MDS rates also increased with decreasing distance from the hypocenter in both Nagasaki and LSS cohort, and with increasing estimated dose in the LSS cohort.

When sex and age at exposure were adjusted, Cox analysis showed that the MDS incidence rate was significantly and inversely related to the exposure distance in the NOC. Analyses of the LSS cohort also revealed that dose was a strong risk factor for MDS. Effects of exposure distance and dose on MDS were observed in both high- and low-risk MDS in both cohorts (Fig 2a, b). Through these analyses, it was suggested that the dose of radiation and distance from the hypocenter had stronger effects on high-risk MDS than low-risk MDS. In terms of the effect of age at exposure, when we adjusted for attained age in 1985 in the NOC, age-specific MDS risks was higher in the young age group; with risks for those born after 1925 is about 1.75 (95% CI, 1.05–2.90) times as those born in earlier years. The fitted distance-response curve is shown in Fig. 6. It was a linear relationship (ERR 4.3 at 1 Gy).

Hematological disorders as long-term effects of atomic bomb radiation

In the analyses using "Open City cohort" of Hiroshima and Nagasaki, and the LSS cohort, it is clear that radiation by atomic bomb caused several hematological neoplasms as late effects. The increase of leukemia was noticed some years after exposure and it was evident at least 5 years after exposure. Dose had a significant relationship with the risk of leukemia and MDS, clearly demonstrating that radiation is a causative factor for both leukemia and MDS. However, considering the effects of sex, age at exposure, and attained age on the risks for these diseases, there would be very complex biological mechanisms of how hematological neoplasms develop after radiation exposure.

The risk of MDS increased at least 45 years after exposure, and also the relative risk of AML mortality. These data raise the suggestion that the risk of hematological neoplasms could be lifelong for survivors. So far, there is no clear explanation how the effect of atomic bomb lasts for such a long period. Considering the hierarchy of hematopoiesis, it is assumed that only hematopoietic stem cells could hold the effects of radiation for such a long time, however, this hypothesis needs to be examined. Epidemiological data from "Open City cohort" of Hiroshima and Nagasaki, and the LSS cohort have been providing pivotal data of the effects of radiation on human, which will and has very important sources. Because the possibility of the life-long risk for hematological neoplasms among survivors, which was suggested by recent works, highlighted the necessity of the continuing follow-up of both cohorts.

Conflict of interest None.

References

1. Folley JH, Borges W, Yamasaki T. Incidence of leukemia in the atomic bomb survivors of Hiroshima and Nagasaki, Japan. Am J Med. 1952;13:311–21.
2. Ishimaru T, Hoshino T, Ichimaru M, Okada H, Tomiyasu T. Leukemia in atomic bomb survivors, Hiroshima and Nagasaki, 1 (October), pp. 1950–30, September 1966. Radiat Res. 1971;45(1): 216–33.
3. Ichimaru M, Ishimaru T, Belsky JH. Incidence of leukemia in Atomic bomb survivors belonging to a fixed cohort in Hiroshima and Nagasaki, 1950–71. Radiation dose, years after exposure, age at exposure, and type of leukemia. J Radiat Res. 1978;19:262–82.

4. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposals for the classification of the acute leukemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33:451–8.
5. Matsuo T, Tomonaga M, Bennett JM, Kuriyama K, Imanaka F, Kuramoto A, et al. Reclassification of leukemia among A-bomb survivors in Nagasaki using French-American-British (FAB) classification for acute leukemia. Jpn J Clin Oncol. 1988;18:91–6.
6. Tomonaga M, Matsuo T, Carter RL, et al. Differential effects of atomic bomb irradiation in inducing major leukemia types: analyses of open-city cases including the Life Span Study cohort based upon update diagnostic systems and the dosimetry system 1986 (DS86). RERF TR 9-91. 1993.
7. Kamada N, Tanaka K, Oguma N, Mabuchi K. Cytogenetic and molecular changes in leukemia among atomic bomb survivors. J Radiat Res (Tokyo). 1991;32(Suppl 2):257–65.
8. Preston DL, Kusumi S, Tomonaga M, et al. Cancer incidence in atomic-bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950–1987. Radiat Res. 1994;137:S68–97.
9. Richardson D, Sugiyama H, Nishi N, Sakata R, Shimizu Y, Grant EJ, Soda M, Hsu WL, Suyama A, Kodama K, Kasagi F. Ionizing radiation and leukemia mortality among Japanese Atomic Bomb Survivors, 1950–2000. Radiat Res. 2009;172:368–82.
10. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51:189–99.
11. Iwanaga M, Hsu WL, Soda M, Takasaki Y, Tawara M, Joh T, Amenomori T, Yamamura M, Yoshida Y, Koba T, Miyazaki Y, Matsuo T, Preston DL, Suyama A, Kodama K, Tomonaga M. Risk of myelodysplastic syndromes in people exposed to ionizing radiation: a retrospective cohort study of Nagasaki atomic bomb survivors. J Clin Oncol. 2011;29:428–34.

Cancer Science
The official journal of the Japanese Cancer Association JCA

Long-term outcome following imatinib therapy for chronic myelogenous leukemia, with assessment of dosage and blood levels: the JALSG CML202 study*

Kazunori Ohnishi,[1,17] Chiaki Nakaseko,[2] Jin Takeuchi,[3] Shin Fujisawa,[4] Tadashi Nagai,[5] Hirohito Yamazaki,[6] Tetsuzo Tauchi,[7] Kiyotoshi Imai,[8] Naoki Mori,[9] Fumiharu Yagasaki,[10] Yasuhiro Maeda,[11] Noriko Usui,[12] Yasushi Miyazaki,[13] Koichi Miyamura,[14] Hitoshi Kiyoi,[15] Shigeki Ohtake,[16] Tomoki Naoe[15] and for the Japan Adult Leukemia Study Group

[1]Oncology Center, Hamamatsu University School of Medicine, Hamamatsu; [2]Department of Hematology, Chiba University Hospital, Chiba; [3]Department of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo; [4]Department of Hematology, Yokohama City University Medical Center, Yokohama; [5]Division of Hematology, Jichi Medical University Hospital, Shimotsuke; [6]Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science, Kanazawa; [7]Department of Hematology, Tokyo Medical University, Tokyo; [8]Department of Hematology, Institute for Artificial Organs, Transplantation & Gene Therapy, Sapporo Hokuyu Hospital, Sapporo; [9]Department of Hematology, Tokyo Women's Medical University School of Medicine, Tokyo; [10]Department of Hematology, International Medical Center, Saitama Medical University, Hidaka; [11]Department of Hematology, Kinki University Faculty of Medicine, Osakasayama; [12]Division of Oncology and Hematology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo; [13]Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [14]Hematology Division, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya; [15]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya; [16]Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

(Received December 2, 2011/Revised February 20, 2012/Accepted February 20, 2012/Accepted manuscript online February 25, 2012)

A prospective multicenter Phase II study was performed to examine the efficacy and safety of imatinib therapy in newly diagnosed Japanese patients with chronic-phase CML. Patients were scheduled to receive imatinib 400 mg daily. Plasma imatinib concentrations were measured by liquid chromatography–tandem mass spectrometry. In 481 evaluable patients, estimated 7-year overall survival (OS) and event-free survival (EFS) at a median follow-up of 65 months were 93% and 87%, respectively. Because imatinib dosage was reduced in many patients due mainly to adverse events, subgroup analysis was performed according to the mean daily dose during the first 24 months of treatment: ≥360 mg (400-mg group; *n* = 294), 270–359 mg (300-mg group; *n* = 90) and <270 mg (200-mg group; *n* = 67). There were no significant differences in OS and EFS between the 300- and 400-mg groups; however, cumulative rates of complete cytogenetic and major molecular responses differed significantly between the two groups. There were no significant differences in mean imatinib trough levels between these two groups for the patients in whom trough levels had been measured. Survival and efficacy in the 200-mg group were markedly inferior to the former two groups. These results suggest that, although a daily dose of 400 mg imatinib is associated with better outcomes, 300 mg imatinib may be adequate for a considerable number of Japanese patients who are intolerant to 400 mg imatinib. Blood level monitoring would be useful to determine the optimal dose of imatinib. (*Cancer Sci*, doi: 10.1111/j.1349-7006.2012.02253.x, 2012)

Imatinib mesylate, a selective BCR-ABL1 kinase inhibitor, has demonstrated remarkable long-term efficacy in the treatment of chronic-phase (CP) CML[1] and now is the standard therapy for this disease.[2] An 8-year follow-up during the International Randomized Study of Interferon and STI571 (IRIS) on newly diagnosed CP CML demonstrated that continuous imatinib therapy exhibited superior efficacy and improved survival.[3] In Japan, imatinib was approved for the treatment of CML in 2001, and a multicenter prospective Phase II study of imatinib therapy (CML202 study) for newly diagnosed CP CML was immediately initiated by the Japan Adult Leukemia Study Group (JALSG). Herein, we report on the results of this study after a median follow-up period of 65 months.

In the present study, although the daily dose of imatinib was set at 400 mg, because of adverse events in many patients the dosage was reduced to less than 400 mg. Nevertheless, the overall efficacy and outcomes were excellent compared with that reported in other studies.[1,4,5] The relatively smaller body size of Japanese patients may explain why a daily dose of < 400 mg imatinib was adequate in some patients.[6] To confirm this assumption, we measured plasma trough levels of imatinib in patients receiving 400 or 300 mg imatinib daily and evaluated the association between plasma concentrations of imatinib and the efficacy, as well as long-term outcome, in these patients.

Materials and Methods

Study design and treatment. The present study was a prospective multicenter Phase II study on previously untreated, newly diagnosed patients with CP CML, with patients receiving a daily dose of 400 mg imatinib. The primary endpoint was overall survival (OS). Secondary endpoints included the rate of a complete hematologic response (CHR), the rate of a cytogenetic response, progression-free survival (PFS), event-free survival (EFS), and safety. The study was registered with the UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index/htm, accessed 10 Sep 2005; registration no. C000000153, the JALSG CML202 study).

Patients. Patients were eligible for inclusion in the study if they were 15 years or older, had de novo Philadelphia (Ph)-chromosome positive CP CML and had not received interferon-α treatment for CML. Further eligibility criteria were adequate liver function (serum bilirubin level ≤2.0 mg/dL and serum liver aminotransferase less than threefold the upper limit of normal), kidney function (serum creatinine ≤2.0 mg/dL), heart and lung function, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–3, and no prior

[17]To whom correspondence should be addressed.
E-mail: kohnishi@hama-med.ac.jp
*Name of trial register: JALSG CML202. Registration no. C000000153, UMIN Clinical Trials Registry.

doi: 10.1111/j.1349-7006.2012.02253.x

or concurrent malignancy. Written informed consent was obtained from all patients prior to registration. The study protocol was reviewed and approved by the institutional review board of all the participating centers and the study was conducted in accordance with the Declaration of Helsinki.

Dose modification of imatinib. Patients were scheduled to receive imatinib at an oral daily dose of 400 mg. Lower dose of < 400 mg daily were permitted at the start of imatinib therapy in patients who were old and/or had a small body size, but it was planned to increase the dose of imatinib to 400 mg within the first month if patients tolerated the reduced dose. Dose escalation to 600 mg was implemented if patients failed to achieve a complete hematologic response (CHR) at 3 months or a major cytogenetic response at 6 months in the absence of dose-limiting adverse events. If patients did not exhibit a CHR at 6 months, they were switched to alternative therapy. If patients achieved a major cytogenetic response within 9 months, imatinib at 400 mg or the adjusted dose was maintained until disease progression.

If Grade 2 non-hematologic toxicities occurred and did not resolve spontaneously, imatinib was interrupted until the toxicities had been ameliorated to Grade 1 or less, and then resumed at the preceding dose. If Grade 3 or 4 non-hematologic or hematologic toxicities occurred, imatinib was interrupted until the toxicities had been ameliorated to Grade 1 or less, and then resumed at a reduced daily dose of 300 mg. Imatinib therapy was discontinued in the event of failure to achieve a CHR at 6 months, intolerance to imatinib, or disease progression to an accelerated phase (AP) or blast crisis (BC).

Definitions. The phases of CML (i.e. CP, AP, or BC) were defined as described previously in the IRIS study.[7] A CHR was defined as a reduction in the leukocyte count to $<10 \times 10^9$/L and a reduction in the platelet count to $<450 \times 10^9$/L that persisted for at least 4 weeks. Cytogenetic responses were evaluated by G-banding of at least 20 marrow cells in metaphase and were categorized as complete (CCyR; no cells positive for the Ph chromosome) and partial (PCyR; 1–35% of cells positive for the Ph chromosome). A major cytogenetic response (MCyR) was defined as complete or partial responses.[2] A major molecular response (MMR) was defined as a 3-log reduction or more in *BCR-ABL1* transcripts compared with median baseline levels, as measured by reverse-transcription real-time quantitative polymerase chain reaction (RQ-PCR)[8,9] or the transcription-mediated amplification and hybridization protection assay (TMA-HPA)[10,11] (For details, refer to Fig. S1 and Data S1, which are available as online Supplementary Material for this paper).

Event-free survival was defined as the time between registration and the earliest occurrence of any of the following events: death due to any cause, progression to AP or BC, and/or loss of MCyR or CHR. Progression-free survival was defined as the time between registration and the earliest occurrence of any of the following events: death due to any cause or progression to AP or BC. Overall survival was defined as the time between the date of registration and death due to any cause. Hematopoietic stem cell transplantation (HSCT) was not censored. Adverse events were assessed according to the National Cancer Institute–Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm, accessed 15 Mar 2012). The mean daily dose of imatinib in a designated period was defined as the total of the doses administered divided by the total number of days on which it was administered.

Measurement of trough plasma levels of imatinib. Blood samples were obtained within 24 ± 2 h after the last imatinib administration from patients who had been receiving 300 or 400 mg imatinib daily without any dose modification for at least 2 years. Plasma was immediately separated at 4°C and at 5000*g* for 10 min by centrifugation and stored at −80°C until measurement. Plasma imatinib concentrations were measured at the Toray Research Center (Tokyo, Japan), as reported previously.[12] Briefly, sample extracts were analyzed using reverse-phase chromatography with a Waters Symmetry column (Waters, Milford, MA, USA), followed by detection with a Sciex API 3000 mass spectrometer (PE Biosystems, Foster City, CA, USA). The lower limit of quantification was 4 ng/mL imatinib mesylate and the assay was fully validated. The precision from validation ranged from 99 ± 5% to 108 ± 5% over the concentration range 4–10 000 ng/mL.[13] The internal standard, imatinib mesylate, was provided by Novartis Pharma (Basel, Switzerland) and the assay system was approved by Novartis Pharma.

Statistical analysis. The Kaplan–Meier method and 95% confidential intervals (CI) were used to analyze OS, PFS, and EFS. Differences between subgroups of patients were evaluated using the log-rank test. Cumulative rates of CHR and cytogenetic responses were estimated according to the competing risk method, in which discontinuation of imatinib was evaluated as competing risk. Comparisons of baseline characteristics in the subgroups were made using the chi square test or Fisher's exact test for categorical variables, and with the Mann–Whitney *U*-test for continuous variables. All statistical analyses were performed using JMP software (SAS Institute, Cary, NC, USA) and R software (http://www.r-project.org, accessed 15 Feb 2011). Two-sided $P < 0.05$ was considered significant.

Results

Patients. Between April 2002 and April 2006, 489 patients from 86 hospitals belonging to the JALSG were enrolled in the CML202 study. Of these patients, three were deemed to be ineligible for inclusion because they were in AP, and a further five were excluded because of insufficient data. The characteristics of the remaining 481 evaluable patients at the time of registration are given in Table 1. The median follow-up time was 65.2 months (range 0.4–95.1 months). Eighty-two of 481 patients (17%) discontinued imatinib therapy or were switched to other therapy (Table 2).

Efficacy. For all 481 evaluable patients, the estimated cumulative rate of CHR was 96% at 7 years, whereas the rates for MCyR and CCyR were 94% and 90%, respectively (Fig. 1a). The *BCR-ABL1* transcript was measured in 428 patients using TMA-HPA and/or RQ-PCR. Levels of the *BCR-ABL1* transcript decreased to <100 copies/μg mRNA (i.e. MMR) in 39% of patients at 18 months and in 79% of patients after 7 years from the start of imatinib (Fig. 1b). According to the Sokal scoring system,[14] the cumulative rates of CCyR were 93%, 84%, and 82% in the low-, intermediate-, and high-risk groups, respectively. There was a significant difference in the rates of CCyR between the low- and intermediate/high-risk groups ($P = 0.006$).

Long-term outcomes. The estimated 7-year rates (with 95% CI) of OS, PFS, and EFS were 93% (90–96%), 93% (90–95%), and 87% (84–91%), respectively (Fig. 1c). The estimated rate of freedom from progression to AP/BC was 97% (95% CI 96–99%) and the estimated 7-year rates of OS according to the Sokal scoring system for patients in the low-, intermediate-, and high-risk groups were 95%, 90%, and 91%, respectively. Patients in the low-risk group exhibited significantly better OS ($P = 0.016$) and EFS ($P = 0.022$) than those in the intermediate- or high-risk groups. In the landmark analysis, patients who had achieved a CCyR at 12 months or an MMR at 18 months exhibited significantly better PFS than

doi: 10.1111/j.1349-7006.2012.02253.x

Table 1. Patient characteristics

Total no. patients	489
No. evaluable patients	481
Age (years)	52 (15–88)
No. patients ≥60 years of age (%)	141 (29)
Sex (M/F, %)	310/171 (64/36)
ECOG PS	
0	441 (92)
1	36 (8)
2	4 (1)
3	0 (0)
Duration from diagnosis (months)	0.4 (0–8.3)
Sokal risk group (%)	
Low	253 (53)
Intermediate	163 (34)
High	65 (14)
Hasford risk group (%)	
Low	202 (42)
Intermediate	227 (47)
High	39 (8)
Unknown	13 (3)
Additional chromosomal abnormalities (%)	
Yes†	51 (11)
Trisomy 8	4 (0.8)
Double Ph	3 (0.6)
Loss of sex chromosome	3 (0.6)
Others	41 (8.5)
Splenomegaly (%)	
Yes	127 (27)
≥10 cm below the costal margin	29 (6)
WBC ($\times 10^9$/L)	36.7 (4.5–634.7)
Hb (g/dL)	12.9 (4.8–19.1)
Platelets ($\times 10^9$/L)	473 (96–2916)
PB blast (%)	0 (0–13.0)
PB basophils (%)	5.0 (0–19.0)
Body weight (kg)	
All patients	61.8 ± 12.1
Men	66.9 ± 10.9
Women	52.6 ± 8.2
BSA (m^2)	
All patients	1.621 ± 0.187
Men	1.714 ± 0.148
Women	1.453 ± 0.121

Data are presented as the mean ± SD, as the median with the range given in parentheses, or as the number of patients in each group with percentages given in parentheses, as appropriate. †The presence of additional chromosomal abnormalities was not an exclusion criterion for the present study. BSA, body surface area; ECOG PS, Eastern Cooperative Oncology Group performance status; Hb, hemoglobin; PB, peripheral blood; WBC, white blood cells.

Table 2. Patients' treatment status

	No. patients (%)
Continued imatinib treatment	399 (83.0)
Discontinued imatinib treatment	82 (17.0)
Reasons for discontinuation and/or change in therapy	
Adverse events	34 (7.1)
Disease progression	11 (2.3)
Unsatisfactory therapeutic effect	12 (2.5)
HSCT	6 (1.2)
Death	2 (0.4)
Lost to follow-up	7 (1.5)
Withdrawal of consent	8 (1.7)
Unknown	2 (0.4)

HSCT, hematopoietic stem cell transplantation.

those without CCyR or MMR ($P = 0.0005$ and $P = 0.012$, respectively).

Safety. The adverse events observed in all patients are listed in Table 3. Grade 3 or 4 hematologic adverse events were neutropenia (18%), thrombocytopenia (12%), and anemia (6%). Grade 3 or 4 non-hematologic adverse events included skin eruption (8%) and peripheral edema (0.6%). Grade 3 or 4 liver dysfunction was reported in 4% of patients. Congestive heart failure (Grade 3) developed in one patient and interstitial pneumonitis (Grade 3) developed in another patient. Grade 3 or 4 thrombocytopenia and skin eruptions occurred more frequently in the present study than in the IRIS study.[7]

Efficacy and outcomes in relation to imatinib dosage. Although it was planned to administer imatinib to patients at a dose of 400 mg daily, 82 patients (17%) discontinued imatinib or were switched to other treatment mainly because of adverse events or unsatisfactory efficacy (Tables 2, 3). Dose reduction or interruption were required in 223 (46%) patients, with escalated doses given to 10 patients (2%) during the first 24 months. Among all 481 patients, the initial dose of imatinib was 400 mg in 458 patients (95.2%), 300 mg in 10 patients (2.1%), 200 mg in 11 patients (2.3%), 100 mg on one patient, and 600 mg in one patient. The mean daily dose during the first 24 months of treatment was ≥360 mg in 294 patients (61%; designated the "400-mg group"), 270–359 mg in 90 patients (19%; designated the "300-mg group"), and < 270 mg in 67 patients (14%; designated the "200-mg group"). Thirty patients (6%) discontinued imatinib during the first 24 months. Regarding the safety profile, Grade 3 or 4 neutropenia, thrombocytopenia, liver dysfunction, and skin eruptions tended to be observed more frequently in the 300- and 200-mg groups because dose reductions from the scheduled dose of 400 mg imatinib daily were mostly made for patients in these groups because of adverse events (Table 3). The patients in the 300-mg group were significantly more likely to be female, older, have a lower body weight (BW), and a smaller body surface area (BSA) than patients in the 400-mg group (Table 4). Patients in the 300- and 200-mg groups had significantly higher Sokal risk than patients in the 400-mg group ($P = 0.001$). Of the patients in the 400- and 300-mg groups, age ($P = 0.0024$) and sex ($P = 0.0077$) were significant independent predictors for OS, as determined by multivariate analysis; however, dosage was not a significant predictor of OS ($P = 0.64$).

Efficacy and survival were analyzed according to the mean daily dose during the first 6, 12, and 24 months. During each period, the estimated cumulative rate of CCyR or MMR was significantly higher for patients in the 400- and 300-mg groups than for patients in the 200-mg group ($P < 0.001$ and $P < 0.0001$, respectively). There was a significant difference in achieving CCyR or MMR between the 400- and 300-mg groups ($P = 0.018$ and $P = 0.017$, respectively; Fig. 2a,b). There were no significant differences in OS and EFS between the 400- and 300-mg groups during the first 24 months ($P = 0.77$ and $P = 0.49$, respectively). However, the OS and EFS of the 200-mg group were significantly inferior to those of the 400- and 300-mg groups during the same periods ($P = 0.009$ and $P = 0.002$, respectively; Fig. 3a,b). Survival was analyzed according to the mean daily dosage of imatinib during the first 24 months per BW (Table 5). Patients who received a mean dose of imatinib per BW that was >5.0 mg/day/kg showed significantly superior OS and EFS than those receiving ≤5.0 mg/day/kg ($P = 0.0012$ and $P = 0.0016$, respectively; Fig. 4). These results indicate that patients who had relatively high daily dosage per BW had better OS and EFS, although the actual daily dose had been lower than 400 mg imatinib.

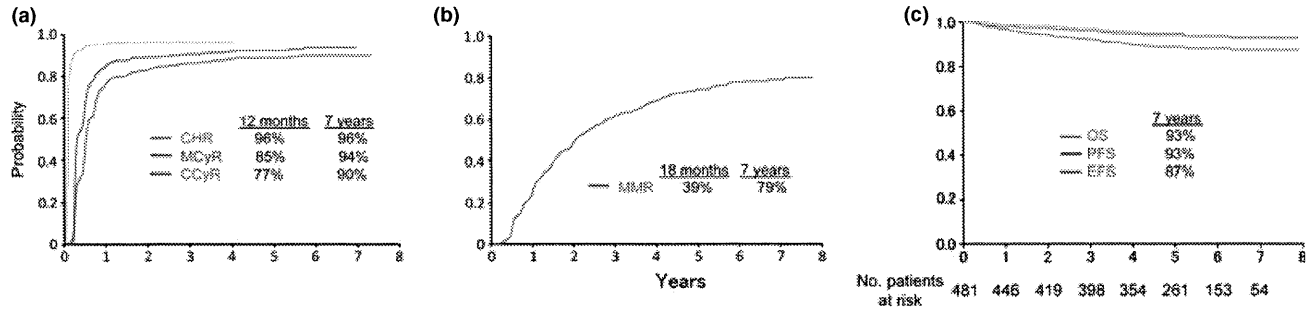

Fig. 1. Cumulative best (a) cytogenetic and (b) molecular responses and (c) survival of patients on imatinib therapy for chronic phase CML. Cumulative rates of responses were estimated according to the competing risk method. Discontinuation of imatinib was evaluated as a competing risk. CHR, complete hematologic response; MCyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response; OS, overall survival; PFS, progression-free survival; EFS, event-free survival.

Table 3. Adverse events associated with imatinib therapy

Adverse event†	No. patients (%)				
	All patients (n = 481)		400-mg group‡ (n = 294)	300-mg group‡ (n = 90)	200-mg group‡ (n = 67)
	All grades	Grade 3 or 4	Grade 3 or 4	Grade 3 or 4	Grade 3 or 4
Non-hematologic					
Superficial edema	234 (48.6)	3 (0.6)	0	3 (3.3)	0
Nausea/vomiting	106 (22.0)	4 (0.8)	2 (0.7)	1 (1.1)	1 (1.5)
Anorexia	94 (19.5)	5 (1.0)	2 (0.7)	2 (2.2)	1 (1.5)
Muscle cramps	81 (16.8)	1 (0.2)	0	1 (1.1)	0
Musculoskeletal pain (myalgia)	100 (20.8)	5 (1.0)	2 (0.7)	0	2 (3.0)
Arthralgia	47 (9.8)	1 (0.2)	0	0	0
Rash	192 (39.9)	37 (7.7)	7 (2.4)	10 (11.1)	14 (20.9)
Fatigue	114 (23.7)	0 (0)	0	0	0
Diarrhea	75 (15.6)	2 (0.4)	1 (0.3)	0	0
Headache	36 (7.5)	1 (0.2)	0	0	0
Hemorrhage	24 (5.0)	3 (0.6)	2 (0.7)	0	1 (1.5)
Pyrexia	49 (10.0)	1 (0.2)	1 (0.3)	0	0
Depression	25 (5.2)	0 (0)	0	0	0
Infection	35 (7.3)	8 (1.7)	5 (1.7)	0	2 (3.0)
Interstitial pneumonitis	3 (0.6)	1 (0.2)	0	0	1 (1.5)
Hematologic					
Anemia	197 (41.0)	28 (5.8)	12 (4.1)	4 (4.4)	10 (14.9)
Neutropenia	188 (39.1)	85 (17.7)	36 (12.2)	25 (27.8)	18 (26.9)
Thrombocytopenia	199 (41.4)	59 (12.3)	19 (6.5)	20 (22.5)	16 (23.9)
Biochemical					
Elevated ALT/AST	99 (20.6)	18 (3.7)	3 (1.0)	6 (6.7)	7 (10.4)
Renal dysfunction	37 (7.7)	1 (0.2)	1 (0.3)	0	0

†Adverse events were assessed according to the National Cancer Institute–Common Toxicity Criteria version 2.0. ‡Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and < 270 mg imatinib, respectively. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Plasma trough levels of imatinib according to the daily dose. Plasma trough levels (C_{min}) of imatinib were determined in 50 patients who continuously received imatinib at a daily dose of 300 mg (n = 24) or 400 mg (n = 26) without any dose modification (Table 6). The patients receiving 300 mg imatinib tended to be older and to have a smaller BSA than patients in the 400-mg group. These tendencies did not different from those of the entire study population (Tables 4 and 6). There was no significant difference in mean C_{min} between the two groups (P = 0.673). The C_{min} in 15 of 24 patients (63%) receiving 300 mg imatinib and in 15 of 26 patients (58%) receiving 400 mg imatinib were distributed above 1000 ng/mL, and the ratio of patients >1000 ng/mL C_{min} did not differ significantly between the two groups (P = 0.10). However, the C_{min} in patients receiving 300 mg imatinib was distributed towards lower concentrations compared with those receiving 400 mg imatinib. There was a significant correlation between C_{min} and age only in the 400-mg group (P = 0.034), with weak correlations between C_{min} and BW or BSA. These results indicate that small, elderly, and/or female patients receiving 300 mg imatinib daily had almost the same C_{min} as patients receiving 400 mg daily.

Discussion

In the present study (CML202), the best cumulative rates of MCyR and CCyR 7 years after the start of imatinib were 94% and 90%, respectively, and the estimated 7-year OS and EFS

doi: 10.1111/j.1349-7006.2012.02253.x

Table 4. Patient characteristics in each of the mean daily dose groups during the first 24 months of treatment

	Imatinib daily dose group†				*P*-value
	400 mg	300 mg	200 mg	Discontinued	
No. patients	294	90	67	30	
Daily dose (mg)	398 ± 17	310 ± 23	187 ± 68	NA	
No. men/women	212/82	46/44	30/37	22/8	<0.0001
Age (years)	48 (16–81)	57 (19–79)	63 (19–87)	52.5 (15–88)	<0.0001
Body weight (kg)	64.6 ± 11.8	57.6 ± 10.5	55.3 ± 10.0	61.8 ± 15.3	<0.0001
BSA (m^2)	1.67 ± 0.18	1.55 ± 0.16	1.51 ± 0.17	1.61 ± 0.22	<0.0001
Sokal risk group (*n*)					
Low	180	39	23	11	<0.0001
Intermediate	84	30	32	13	
High	30	21	12	6	
Dose reduction (*n*)	1	69	59	NA	
Interruption (*n*)	65	21	8	NA	
Dose escalation (*n*)	10	0	0	NA	

Unless indicated otherwise, data are given as the mean ± SD or as the median with the range given in parentheses. †Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and <270 mg imatinib, respectively. BSA, body surface area; NA, not applicable.

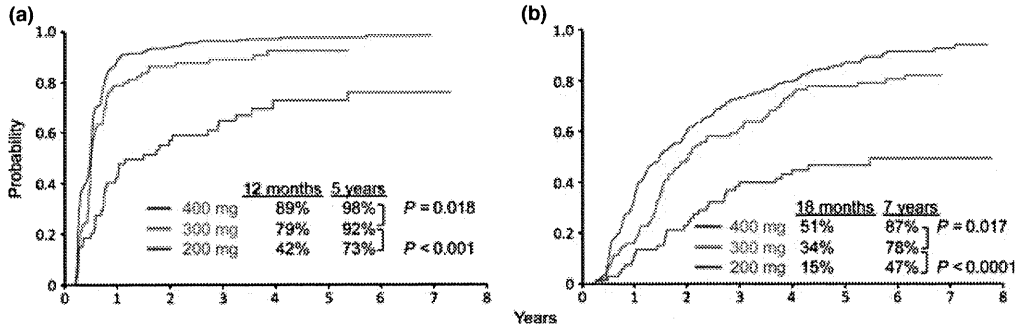

Fig. 2. Cumulative rates of best responses according to the mean daily dose during the first 24 months of treatment with imatinib. (a) Cumulative rates for complete cytogenetic responses (CCyR). (b) Cumulative rates of major molecular responses (MMR). Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and <270 mg imatinib, respectively.

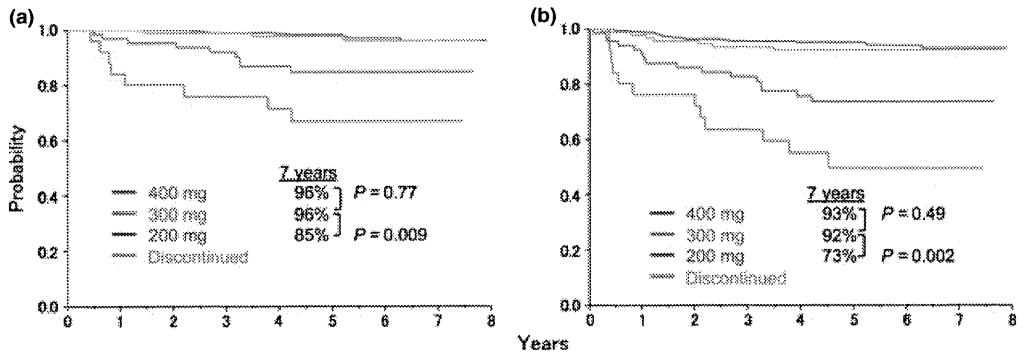

Fig. 3. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months. Mean daily doses in the 400- (*n* = 294), 300- (*n* = 90), and 200-mg (*n* = 67) groups were ≥360, 270–359, and < 270 mg imatinib, respectively.

rates were 93% and 87%, respectively. The Sokal risk showed favorable prognostic significance in low-risk patients compared with intermediate- or high-risk patients. These results are comparable to those reported in the IRIS trial and others studies in Western countries.[3–5] In terms of baseline characteristics, there was a tendency for fewer patients with a high-risk Sokal score in the present study compared with the IRIS study. We believe this is due to the Japanese medical system, in which a considerable number of people undergo annual medical check-ups.

Imatinib is currently established as the first-line therapy for patients with CP CML. Nevertheless, several controversial issues remain,[15] with the dose of imatinib as one of the most important.[6,16–21] In the present study, many patients received a lower dose of imatinib than the planned initial dose of 400 mg. Therefore, we performed subgroup analysis according

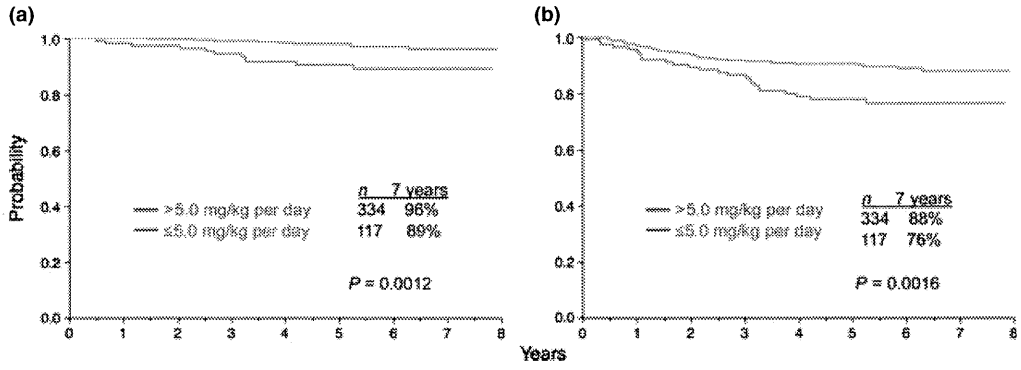

Fig. 4. (a) Overall and (b) event-free survival according to the mean daily dose during the first 24 months per body weight. The cut-off value was set at >5.0 mg/day/kg (e.g. if a patient whose body weight was <60 kg received imatinib at a mean daily dose of 300 mg).

Table 5. Number of patients and survival according to the mean daily dose of imatinib during the first 24 months per body weight

	Mean daily dose/body weight (mg/day/kg)				
	>5.0†		≤5.0		*P*-value
	Actual bodyweight (kg)	No. patients	Actual bodyweight (kg)	No. patients	
Imatinib daily dose group‡					
400 mg	<80	266	≥80	28	
300 mg	<60	63	≥60	27	
200 mg	<40	5	≥40	62	
Estimated 7-year OS	96%		89%		0.0012
Estimated 7-year EFS	88%		76%		0.0016

†The cut-off value was set at >5.0 mg/day/kg (e.g. the mean daily dose of imatinib during the first 24 months (300 mg) divided by body weight [<60 kg]). ‡Mean daily doses in the 400-, 300-, and 200-mg groups were ≥360, 270–359, and <270 mg imatinib, respectively. Patients who discontinued imatinib were not included in the analysis. EFS, event-free survival; OS, overall survival.

to the mean daily dose during the first 6, 12, and 24 months of treatment. The rate of achieving CCyR or MMR differed significantly between the 300- and 400-mg groups during the first 24 months. Even so, there were no significant differences in OS, PFS, and EFS between the 300- and 400-mg groups during the first 6, 12, or 24 months of treatment. Conversely, the 200-mg group showed markedly inferior cytogenetic and/or molecular responses, as well as inferior survival, compared with the 300- and 400-mg groups. We also analyzed outcomes according to the mean daily dosage during the first 24 months per BW, with the results suggesting that patients who had relatively high daily dosage per BW were likely to have better OS and EFS even though the actual daily dose had been lower than 400 mg imatinib. The OS and EFS in the 300-mg group in the present study were not inferior compared with rates reported in the IRIS study (85% at 7 years vs. 83% at 6 years), which suggests that a considerable number of Japanese patients who received doses lower than 400 mg demonstrated an adequate response. A prospective comparative study would be necessary to confirm this observation.

Two recent studies showed a correlation between the plasma trough levels (C_{min}) and response, suggesting that maintaining C_{min} above approximately 1000 ng/mL was associated with improved outcomes.[22,23] In the present study, the mean daily dose was 331 ± 108 mg during the first 24 months and the relatively high dosage of imatinib per BW was associated with better OS and EFS, whereas in the IRIS study the mean daily dose among the patients who continued receiving imatinib was 382 ± 50 mg.[1] On the basis of our results, we assume that the relatively small body size of Japanese patients compared with their Western counterparts may have affected C_{min}, although differences in the metabolism of imatinib because of ethnicity cannot be ruled out either. Therefore, we measured the C_{min} of imatinib in a group of patients who had received imatinib continuously at a daily dose of either 300 or 400 mg. The patients from whom blood samples were collected showed almost similar background characteristics to the entire study population. There was no significant difference in the mean C_{min} between patients receiving 300 or 400 mg imatinib, and there was no significant difference in the ratio of patients whose C_{min} was higher than 1000 ng/mL between the two groups. When pharmacokinetic analyses of patients receiving 400 mg imatinib in the present study are compared with the IRIS study, the C_{min} in the present study was distributed at higher concentrations than in the IRIS study (mean C_{min} 1165 vs. 979 ng/mL, respectively); however, the distribution of C_{min} in patients receiving 300 mg imatinib was similar between the studies.[23] Larson *et al.* reported a weak correlation between C_{min} and age, BW, or BSA in the IRIS study, but also suggested that the effects of body size and age on C_{min} were not likely to be of clinical significance because C_{min} showed large interpatient variability.[23] However, the C_{min} in their female patients was significantly higher than that in male patients, and they speculated that this may be due to the small body size of the female patients. The same tendency was seen in the present study, especially in terms of age and gender. Therefore, a small body size among Japanese old and/or female patients may partly account for the higher C_{min} of imatinib. Regarding

doi: 10.1111/j.1349-7006.2012.02253.x

Table 6. Patient characteristics and plasma trough levels of imatinib according to the daily dose of imatinib

	Imatinib daily dose†		*P*-value
	400 mg	300 mg	
No. patients	26	24	
No. men/women	19/7	12/12	0.092
Age (years)	49 (17–79)	58 (33–76)	0.012
Body weight (kg)	65.2 ± 10.6	59.5 ± 10.7	0.062
BSA (m^2)	1.68 ± 0.17	1.57 ± 0.17	0.034
Sokal risk group (*n*)			
Low	18	13	0.357
Intermediate	6	6	
High	2	5	
C_{min} (ng/mL)			
Mean ± SD	1165 ± 445	1113 ± 426	0.673
Median (range)	1035 (710–2420)	1130 (439–2140)	
% Patients on >1000 ng/mL imatinib	57.7 (15/26)	62.5 (15/24)	0.1
Best response (%)			
MCyR	26 (100)	23 (96)	
CCyR	26 (100)	22 (92)	
MMR	24 (92)	23 (96)	

Unless indicated otherwise, data are given as the mean ± SD, as the median with the range given in parentheses, or as the number of patients in each group with percentages given in parentheses, as appropriate. †Imatinib at a daily dose of 400 or 300 mg without any dose modification. BSA, body surface area; CCyR, complete cytogenetic response; C_{min}, plasma trough level; MCyR, major cytogenetic response; MMR, major molecular response.

the plasma concentration of imatinib in Japanese patients, there are other reports showing sufficient C_{min} in patients receiving imatinib at doses lower than 400 mg,[6,24] but it remains uncertain whether there are any individual or ethnic differences in the metabolism of imatinib.[24,25]

Another possible reason for the satisfactory outcomes seen for patients in the 300-mg group could be that, at this dose, imatinib could be administered continuously to some patients without serious adverse events. A recent study regarding imatinib dosage in Japanese patients reported that, based on multivariate analysis, older age and lower BW are significant risk factors for the discontinuation of imatinib therapy and that patients with these factors were less likely to achieve a CCyR.[18] Continuous and adequate dosage is essential for optimal outcome, and adherence to imatinib therapy is critical.[26,27]

In conclusion, the long-term follow-up of the JALSG CML202 study revealed almost similar excellent outcomes to those of the IRIS study and others. There were no significant differences in OS and EFS between the 300- and 400-mg imatinib groups. However, cumulative rates of cytogenetic or molecular responses in the 300-mg group were inferior to those in the 400-mg group. The results of the present study suggest that imatinib at a dose of 400 mg may be optimal for Japanese patients, but that 400 mg imatinib is not tolerable in a considerable number of patients, and that the measurement of C_{min} is useful in finding the optimal dose, especially in elderly and/or female patients. Nevertheless, excessive dose reductions to <300 mg imatinib should be avoided even in patients who are intolerant to 400 mg imatinib or have a small body size. We hope our findings are useful for the treatment of CML patients in other Asian countries.

Acknowledgments

This work was supported by a grant from the Japanese Ministry of Health, Labour and Welfare. The authors thank the participating doctors and other medical staff of the 86 hospitals who enrolled patients in the present study and provided the necessary data to make the study possible. The authors are indebted to Dr Ryuzo Ohno (Aichi Cancer Center, Nagoya, Japan) for his contribution to the study and in the preparation of manuscript.

Disclosure Statement

KO, YM, HK, and TN received research funding from Novartis. The other authors declare no competing financial interests.

References

1 Druker BJ, Guilhot F, O'Brien SG *et al.* Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 2006; **355**: 2408–17.
2 Baccarani M, Saglio G, Goldman J *et al.* Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood* 2006; **108**: 1809–20.
3 Deininger M, O'Brien S, Guilhot F *et al.* International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *Blood* 2009; **114**: 462 (Abstract).
4 Kantarjian HM, Talpaz M, O'Brien S *et al.* Survival benefit with imatinib mesylate versus interferon-alpha-based regimens in newly diagnosed chronic-phase chronic myelogenous leukemia. *Blood* 2006; **108**: 1835–40.
5 de Lavallade H, Apperley JF, Khorashad JS *et al.* Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. *J Clin Oncol* 2008; **26**: 3358–63.
6 Sakai M, Miyazaki Y, Matsuo E *et al.* Long-term efficacy of imatinib in a practical setting is correlated with imatinib trough concentration that is influenced by body size: a report by the Nagasaki CML Study Group. *Int J Hematol* 2009; **89**: 319–25.
7 O'Brien SG, Guilhot F, Larson RA *et al.* Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003; **348**: 994–1004.
8 Hughes TP, Kaeda J, Branford S *et al.* Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2003; **349**: 1423–32.
9 Gabert J, Beillard E, van der Velden VH *et al.* Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia: a Europe Against Cancer program. *Leukemia* 2003; **17**: 2318–57.
10 Langabeer SE, Gale RE, Harvey RC, Cook RW, Mackinnon S, Linch DC. Transcription-mediated amplification and hybridisation protection assay to determine BCR-ABL transcript levels in patients with chronic myeloid leukaemia. *Leukemia* 2002; **16**: 393–9.
11 Yagasaki F, Niwa T, Abe A *et al.* Correlation of quantification of major bcr-abl mRNA between TMA (transcription mediated amplification) method and real-time quantitative PCR. *Rinsho Ketsueki* 2009; **50**: 481–7. (In Japanese).
12 Bakhtiar R, Lohne J, Ramos L, Khemani L, Hayes M, Tse F. High-throughput quantification of the anti-leukemia drug STI571 (Gleevec) and its main metabolite (CGP 74588) in human plasma using liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002; **768**: 325–40.
13 Champagne MA, Capdeville R, Krailo M *et al.* Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group Phase 1 study. *Blood* 2004; **104**: 2655–60.
14 Sokal JE, Cox EB, Baccarani M *et al.* Prognostic discrimination in "good-risk" chronic granulocytic leukemia. *Blood* 1984; **63**: 789–99.
15 Baccarani M, Cortes J, Pane F *et al.* Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 2009; **27**: 6041–51.
16 Carella AM, Lerma E. Durable responses in chronic myeloid leukemia patients maintained with lower doses of imatinib mesylate after achieving molecular remission. *Ann Hematol* 2007; **86**: 749–52.

17 Sugita J, Tanaka J, Kurosawa M *et al.* Effects of the mean daily doses of imatinib during the first year on survival of patients with chronic myeloid leukemia in Japan: a study of the Hokkaido Hematology Study Group. *Eur J Haematol* 2008; **80**: 160–3.
18 Kanda Y, Okamoto S, Tauchi T *et al.* Multicenter prospective trial evaluating the tolerability of imatinib for Japanese patients with chronic myelogenous leukemia in the chronic phase: does body weight matter? *Am J Hematol* 2008; **83**: 835–9.
19 Nagai T, Takeuchi J, Dobashi N *et al.* Imatinib for newly diagnosed chronic-phase chronic myeloid leukemia: results of a prospective study in Japan. *Int J Hematol* 2010; **92**: 111–7.
20 Hughes TP, Branford S, White DL *et al.* Impact of early dose intensity on cytogenetic and molecular responses in chronic-phase CML patients receiving 600 mg/day of imatinib as initial therapy. *Blood* 2008; **112**: 3965–73.
21 Cortes JE, Baccarani M, Guilhot F *et al.* Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study. *J Clin Oncol* 2010; **28**: 424–30.
22 Picard S, Titier K, Etienne G, *et al.* Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood* 2007; **109**: 3496–9.
23 Larson RA, Druker BJ, Guilhot F *et al.* Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. *Blood* 2008; **111**: 4022–8.
24 Takahashi N, Miura M, Scott SA *et al.* Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response among patients with chronic phase chronic myeloid leukemia. *J Hum Genet* 2010; **55**: 731–7.
25 Soverini S, Angelini S, Barnett M *et al.* Association between imatinib transporters and metabolizing enzymes genotype and response in newly diagnosed chronic myeloid leukemia (CML) patients. *J Clin Oncol* 2010; **28**: 500S. Abstract 6554.
26 Marin D, Bazeos A, Mahon FX *et al.* Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol* 2010; **28**: 2381–8.
27 Noens L, van Lierde MA, De Bock R *et al.* Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. *Blood* 2009; **113**: 5401–11.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Correlation between Amp-CMLTM (FUJIREBIO Inc., Tokyo, Japan) and Fusion Quant M-BCRTM (Ipsogen, Marseille, France).

Data S1. Measurement of major *BCR-ABL1* transcript.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

doi: 10.1111/j.1349-7006.2012.02253.x

original article

Annals of Oncology 22: 1865–1871, 2011
doi:10.1093/annonc/mdq673
Published online 2 February 2011

A prospective dose-finding trial using a modified continual reassessment method for optimization of fludarabine plus melphalan conditioning for marrow transplantation from unrelated donors in patients with hematopoietic malignancies

S. Terakura[1]*, Y. Atsuta[2], M. Sawa[3], H. Ohashi[4], T. Kato[3], S. Nishiwaki[5], N. Imahashi[5], T. Yasuda[5], M. Murata[1], K. Miyamura[5], R. Suzuki[2], T. Naoe[1], T. Ito[3] & Y. Morishita[6] for the Nagoya Blood and Marrow Transplantation Group

[1]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine;* [2]*Department of Hematopoietic Stem Cell Transplantation Data Management, Nagoya University School of Medicine, Nagoya;* [3]*Department of Hematology, Anjo Kosei Hospital, Anjo;* [4]*Clinical Research Center, National Hospital Organization Nagoya Medical Center;* [5]*Department of Hematology, Japanese Red Cross Nagoya First Hospital, Nagoya;* [6]*Department of Hematology and Oncology, JA Aichi Konan Kosei Hospital, Konan, Japan*

Received 10 June 2010; revised 25 September 2010; accepted 21 October 2010

Background: Because of the less graft-facilitating effect by bone marrow (BM), we need to assess a dosage of conditioning more accurately particularly in combination with reduced-intensity conditioning. Thus we examined that modified continual reassessment method (mCRM) is applicable for deciding appropriate conditioning of allogeneic BM transplantation.

Patients and methods: The conditioning regimen consisted of i.v. fludarabine (125 mg/m^2) plus an examination dose of i.v. melphalan. The primary endpoint was a donor-type T-cell chimerism at day 28 with successful engraftment defined as >90% donor cells. Five patients per dose level were planned to be accrued and chimerism data were used to determine the next dose.

Results: Seventeen patients were enrolled at doses between 130 and 160 mg/m^2. The dose was changed from 160 to 130 mg/m^2 (second level) after five full-donor chimerisms. With one patient of 0% chimera in the second level, the dose was increased to 135 mg/m^2 (third level). Following five full-donor chimerisms in the third level, the study was complete as projected.

Conclusions: mCRM was shown to be a relevant method for dose-finding of conditioning regimen. The melphalan dose of 135 mg/m^2 was determined as the recommended phase II dose to induce initial full-donor chimerism.

Key words: allogeneic bone marrow transplantation, dose-finding study, modified continual reassessment method, reduced-intensity conditioning

introduction

The development of a reduced-intensity conditioning (RIC) regimen has enabled older patients or those with comorbidities who are not expected to tolerate the toxicity of myeloablative conditioning to be treated with allogeneic hematopoietic stem-cell transplantation (HSCT) [1–4]. The aim of developing these regimens is to reduce early treatment-related mortality (TRM) while these regimens are still achieving hematopoietic and donor-immune cell engraftment to exert a graft-versus-leukemia (GVL) effect [5]. To assure donor engraftment, most studies use granulocyte colony-stimulating factor-mobilized peripheral-blood stem cells (G-PBSC) with substantial numbers of CD34+ cells and T cells [6], while the immunosuppression required for engraftment is usually accomplished with the fludarabine plus a cytotoxic agent or low-dose total-body irradiation [1–3]. Earlier experiences have shown that the risk of rejection after bone marrow transplantation (BMT) is substantially higher than that of peripheral-blood stem cell transplantation (PBSCT) in RIC transplantation [7]. However, PBSCT from unrelated donors is not currently available in Japan, and bone marrow (BM) may be a more suitable graft source to avoid severe graft-versus-host disease (GVHD), which often leads to TRM [8]. Therefore, a better dosage of conditioning is needed to ensure engraftment in RIC with BM.

Although no one has attempted to systematically conduct a phase I study in conditioning regimen, we adopted a systematic phase I approach by using a modified continual reassessment method (mCRM) to more accurately assess the

Correspondence to: Dr S. Terakura, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa, Nagoya, Aichi 466-8550, Japan. Tel: +81-52-744-2145; Fax: +81-52-744-2161; E-mail: tseit@med.nagoya-u.ac.jp

dosage of a conditioning regimen and to minimize the potential risk of graft failure [9–12]. The mCRM was originally a toxicity-based method that has been shown to be feasible in dose-escalating trials and that has been hypothesized to allow a reduction in patients needed to reach the recommended dose compared with the classical phase I trial design (modified Fibonacci method). We selected the fludarabine plus melphalan regimen that has sufficient potential to induce BM engraftment if melphalan is used at 180 mg/m^2 [13], and further optimization of the melphalan dosage is planned. The rationale for modulating the dose of melphalan resulted from accumulated observations that melphalan alone is associated with toxic effects, while fludarabine is not [14]. Because the optimal dose of melphalan to minimize regimen-related toxic effects (RRTs) and to enable sustained engraftment remains unknown to date, we investigated the recommended dose of melphalan as a phase I study. We showed the recommended dose of melphalan for a future phase II study.

patients and methods

patient and donor eligibility

The potential confounding factors that can influence engraftment efficiency were controlled by eligibility criteria as follows. Eligibility criteria included patients with hematological malignancies who have had more than three courses of chemotherapy, aged between 55 and 65 years or between 40 and 54 years with substantial comorbidity (the hematopoietic cell transplantation comorbidity index (HCT-CI) of ≥1) [15], and no prior stem-cell transplantation. Patients were excluded if they had any of the following: (i) clinically significant infectious disease; (ii) plan to deplete T cells from graft and (iii) severe abnormalities of cardiac, pulmonary, and hepatic functions corresponding to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 3 [16].

All donors were unrelated volunteers recruited through the Japan Marrow Donor Program. Donors were required to be human leukocyte antigen (HLA)-A, -B, and -DRB1 identical or -A, -B identical and -DRB1-1 locus mismatch with their patients based on oligonucleotide typing or DNA sequencing [17, 18]. The protocol was approved by the institutional review boards of all participating institutions. All patients and donors provided written informed consent.

study design and dose determination

To adjust the melphalan dose, we adopted an mCRM that has been hypothesized to allow faster and more accurate dose reduction/escalation in patients needed to reach the recommended dose compared with a classical algorithmic dose modification design [9, 10, 12]. Because the use of fludarabine at 125 mg/m^2 and melphalan at 180 mg/m^2 was known to induce reliable marrow engraftment [13], we set the first dose level to receive 160 mg/m^2 of melphalan. Five patients per dose level were planned to be accrued, and chimerism data were then used to determine the next dose. Each calculation was carried out using software developed by Piantadosi et al. (http://www.cancerbiostats.onc.jhmi.edu/software.cfm, 28 December 2010, date last accessed) [9, 10]. Response (engraftment) in each patient was evaluated by chimerism data at day 28 as following response scores: ≥90% (successful case) = 1; ≥80% to <90% = 0.5; <80% = 0. The dose modification of next level was determined according to the sum of the response scores. Briefly, if the summed response score was 5.0, the melphalan dose of the next level would be reduced; 4.5, no change; <4.0, increased, respectively. A maximum dose reduction of melphalan for the next level was limited to 30 mg/m^2 to avoid the potential risk of graft failure. The recommended melphalan dose for a phase II trial was determined if the calculated dose for the next level was within 5 mg from the prior dose of melphalan.

study endpoints and definitions

Primary endpoint was to achieve the donor-type T-cell chimerism at day 28, with successful engraftment defined as ≥90% donor cells. Secondary endpoints were hematopoietic recovery, RRT within 30 days after transplant, incidence and severity of acute and chronic GVHD, overall survival (OS) and event-free survival (EFS). The day of neutrophil engraftment was defined as the first of the three consecutive days on which the neutrophil count exceeded 500/μl, while platelet engraftment was defined as the first of the three consecutive days when the absolute platelet count exceeded 20 000/μl without platelet infusion. Patients who did not reach neutrophil counts of >500/μl by day 28 after transplantation were considered as having a primary graft-failure. Patients with initial engraftment in whom absolute neutrophil counts declined to <500/μl subsequently were considered to have secondary graft-failure.

conditioning regimens

The conditioning regimen consisted of five doses of fludarabine 25 mg/m^2 administered i.v. on days −6 to −2 combined with two doses of melphalan i.v. on days −3 and −2. Criteria for determination of the melphalan dose are described elsewhere in detail. BM grafts were infused on day 0.

GVHD prophylaxis and grading

GVHD prophylaxis consisted of tacrolimus plus short-course methotrexate [19]. Acute and chronic GVHD were graded by established criteria [20, 21].

chimerism analysis

Serial samples of peripheral-blood mononuclear cells were analyzed for degrees of donor—recipient chimerism using a PCR of informative microsatellite regions after transplantation, as described previously [22]. Samples were routinely analyzed on days 14, 28, 56 and 84, or in cases of disease recurrence or suspicion of graft failure.

statistical analysis

All eligible patients were subjects for analyses of efficacy secondary endpoints. One patient with a protocol violation was excluded for efficacy analyses and GVHD analyses. All patients given conditioning chemotherapy were the subjects for analyses of safety secondary endpoints. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and TRM. For GVHD, death without GVHD; for relapse, death without relapse and for TRM, relapse was the competing event. Curves for EFS and OS were plotted according to the method of a Kaplan-Meier estimate and were compared by the log-rank test [23]. A significance level of $P < 0.05$ was used. Accrual for this study underwent from February 2006 to September 2008, and all data were analyzed as of August 2010. This trial was registered at University hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) System at http://www.umin.ac.jp/ctr/ as C000000325 (28 December 2010, date last accessed). All analyses were conducted using Stata version 10.0 software (Stata Corp., College Station, TX).

results

patient and donor characteristics

Baseline patient and donor characteristics are listed in Table 1. The median age was 58 years (range 42–63). The primary

Table 1. Patient and donor characteristics

Variables	Number
Age, years, median (range)	58 (42–63)
Sex, male/female	10/7
Primary disease	
Acute myeloid leukemia	10
Malignant lymphoma	3
Acute lymphoblastic leukemia	2
Chronic myelogenous leukemia	1
Plasmacytoma	1
Risk of underlying disease	
Advance/standard	11/6
Donor age, years, median (range)	33 (26–48)
Blood type mismatch (match/mismatch)	7/10
Number of infused nuclear cells	
Median (range), 10^8/kg	2.59 (1.52–3.98)
Number of infused CD34+ cells	
Median (range), 10^6/kg	2.31 (1.52–5.45)
Donor–patient HLA compatibility, no. of patients	
6/6	13
5/6[a]	4

Acute leukemia in first complete remission, chronic myelogenous leukemia in first chronic phase, and malignant lymphoma in complete remission were defined as standard risk. All other conditions were defined as advanced risk.

[a]All non-identical pairs were mismatched in HLA-DRB1 in allele level.
HLA, human leukocyte antigen.

diseases were advanced in 11 patients (65%), while HCT-CI at the time of transplantation was 0 in 7 patients, 1 in 5 patients, 2 in 3 patients and 3 in 2 patients. A total of 17 patients were enrolled at doses between 130 and 160 mg/m^2, including one protocol violation for whom cyclosporine A was used as GVHD prophylaxis instead of tacrolimus (patient 2) and one early death (brain hemorrhage, patient 12; Table 2). Both were unavailable in advance for an assessment of the primary endpoint according to the study definition.

The median donor age was 33 years (range 26–48). Donors were HLA-identical (6/6 match) in 13 patients and non-identical (5/6 match) in 4 patients. All non-identical donors were HLA-DRB1 allele mismatch. Because HLA-C typing was not essential for this study enrollment, some data were unavailable. As far as we could tell, all but one donor–patient pair (patient 8) were matched in the HLA-C allele (Table 2). Patient 8 was also mismatched in the HLA-DRB1 allele.

The median infused cell dose was 2.36×10^8/kg (range 0.54 to 3.98×10^8/kg), and the median infused CD34+ cell dose was 2.31×10^6/kg (range 1.52 to 5.45×10^6/kg).

analysis of primary endpoint: conditioning regimen, engraftment and chimerism

The melphalan doses given to patients are summarized in Table 2, and the chimerism analysis data of each leukocyte fraction are depicted (Figure 1A–D). Five consecutive full-donor chimerisms (all were 100% at day 28) were observed in the first level (160 mg/m^2). The summed response score of the first level was 5.0. The melphalan dose for the next level was calculated as 102.5 mg/m^2 using the mCRM program. The upper limitation rule of dose modification was then applied, and the second dose was determined as 130 mg/m^2. In the second level, we observed four patients with 100% chimera and one with 0% chimera at day 28, which eventually resulted in a secondary graft-failure (patient 8). The next melphalan dose following the second level (summed response score = 4.0) was calculated to be 133.1 mg/m^2, which was rounded off to 135 mg/m^2 (third level). In the third level, five consecutive full-donor chimerisms (four 100% and one 90.4%) were observed (summed response score = 5.0). Since the calculated dose for the next level was 130.0 mg/m^2, which was within 5 mg/m^2 of the melphalan dose in the third level in which all patients were successfully engrafted, the study was complete as projected. Overall neutrophil engraftment was achieved in all patients at a median of 14 days (range 12–18) after transplant, which was comparable with the previous data of RIC transplantation, with sustained engraftment achieved in 14 of 15 (93.3%) patients.

Full-donor chimerism had been lost in 2 of 15 assessable patients until day 100. Patient 8 was an HLA-C and -DRB1 allele-mismatched case and consequently developed secondary graft-failure. Patient 8 showed 100% donor chimerism at day 14 but was lost completely at day 28 and developed graft failure. The peripheral leukocyte count was initially recovered on day 15 but gradually fell to 400/μl (100% lymphocyte) by day 25, and BM was severely hypoplastic. Patient 17 achieved 90.4% donor-type T-cell chimerism at day 28 (Figure 1A), then lowered to 75.2% at day 56 and 80.4% at day 84. That patient was diagnosed with a cytogenetic relapse at day 54 and a hematological relapse at day 89. Patient 13 was diagnosed with a hematological relapse at day 84. Thus sustained engraftment at day 100 or until relapse was obtained in 14 of 15 (93%) assessable patients. Continued engraftment at 1 year after transplant was observed among 10 of 10 assessable patients (Table 2). There was no late rejection among enrolled patients.

secondary endpoints

toxicity. Toxic effects of 17 assessable patients within day 28 are graded according to the NCI-CTCAE version 3.0 and summarized in Table 3. Conditioning was generally well tolerated and in concordance with the expected adverse-effect profile of fludarabine plus melphalan conditioning. Grade 3 mucositis, nausea/vomiting and diarrhea were the main toxic effects, affecting 35%, 59% and 24% of the assessable patients, respectively. There was no statistically significant difference in the toxicity grade among dose levels (mucositis, $P = 0.23$; nausea/vomiting, $P = 0.51$; diarrhea, $P = 0.24$; Kruskal-Wallis test). All grade 4 toxic effects were from patient 2 and patient 12. The former developed severe veno-occlusive disease, while patient 12 developed a brain hemorrhage. During their course, they also exhibited severe pulmonary infection (patient 2) and cardiac arrest (patient 12).

GVHD. Of 16 assessable patients, acute GVHD grade I was observed in only one, and the onset was day 22. The cumulative incidence of acute GVHD at day 100 was 6% [95% confidence interval (CI) 0% to 25%]. Five of 13 assessable patients

Table 2. Mel dose, engraftment and chimerism

Patient no.	Disease	Mel dose (mg/m^2)	HLA compatibility A/B/DR	C	Blood type mismatch	Infused cell dose (10^8/kg)	Infused CD34+ dose (10^6/kg)	Day 28 chimerism (%)	Neutrophil engraftment Y/N	Day	Sustained engraftment Y/N	Survival	Outcome/ note
1	NHL	160	6/6	Match	Match	3.87	N.A.	100	Y	14	Y	D (22 m)	
3	AML	160	6/6	N.A.	Match	2.30	N.A.	100	Y	14	Y	D (7 m)	
4	AML	160	6/6	N.A.	Match	3.98	N.A.	100	Y	13	Y	D (18 m)	CNS relapse (9 m)
5	AML	160	5/6	N.A.	Major	0.68	1.52	100	Y	15	Y	D (32 m)	CNS relapse (8 m)
6	Plasmacytoma	160	5/6	N.A.	Match	3.00	N.A.	100	Y	14	Y	D (46 m)	Disease progression
7	AML	130	6/6	Match	Minor	2.32	2.09	100	Y	13	Y	D (37 m)	Relapse (5 m)
8	AML	130	5/6	1mis	Major	0.54	2.31	0	Y	15	N	D	Secondary graft-failure
9	AML	130	5/6	Match	Match	2.40	4.04	100	Y	14	Y	A (40 m+)	
10	NHL	130	6/6	Match	Major	1.18	4.18	100	Y	14	Y	A (38 m+)	
11	AML	130	6/6	Match	Match	1.52	N.A.	100	Y	17	Y	A (38 m+)	
12	NHL	135	6/6	Match	Major	0.77	4.37	N.E.	N.E.	N.E.	N.E.	D	Early death (14 d)
13	AML	135	6/6	Match	Minor	2.78	2.14	100	Y	17	Y	D (5 m)	Relapse (84 d)
14	CML	135	5/6	Match	Minor	3.92	5.45	100	Y	15	Y	A (29 m+)	
15	AML	135	6/6	Match	Major	0.94	2.59	100	Y	18	Y	A (27 m+)	
16	ALL	135	6/6	Match	Match	2.36	2.17	100	Y	12	Y	A (25 m+)	
17	AML	135	6/6	Match	Minor	2.00	2.24	90.4	Y	17	Y	A (22 m+)	Relapse (89 d)

Patient 2 was not listed because of protocol violation.

Mel, melphalan; HLA, human leukocyte antigen; Y, yes; N, no; m, months; d, days; NHL, non-Hodgkin's lymphoma; N.A., not available; D, dead; CNS, central nervous system; 1mis, 1 locus mismatch; A, alive; N.E., not evaluable; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia.

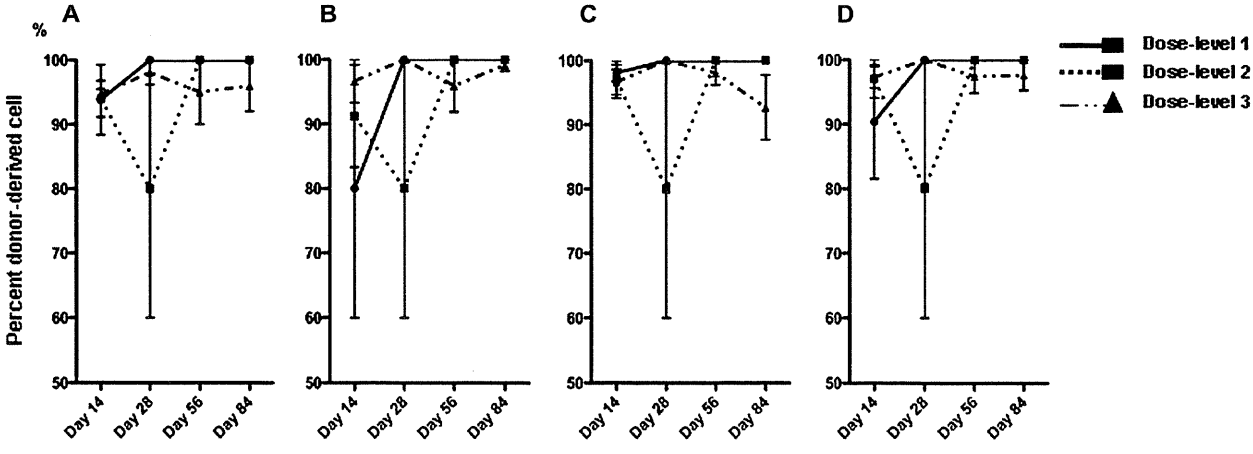

Figure 1. Serial measurement of lineage-specific peripheral blood chimerism. (A) CD3+ T-cell fraction. (B) CD33+ polymorphonuclear cell fraction. (C) CD16+/CD56+ NK-cell fraction. (D) Flowthrough fraction. The mean (±SEM) percentage of donor-derived cells is plotted for each dose-level group. The solid line with filled rectangle represents the first dose level; the dotted line with filled rectangle, the second dose level and the broken line with filled triangle, the third dose level. NK, natural killer; SEM, standard error of the mean.

developed chronic GVHD, with *de novo* onset in all 5 cases (limited type in 2 patients and extensive type in 3), for a 1-year cumulative incidence of chronic extensive GVHD of 31% (95% CI 11% to 54%).

survival. The median follow-up of survivors was 2.6 years (range 1.8–3.8 years), while the OS of eligible patients without a protocol violation (n = 16) was 52% (95% CI 23% to 74%) at 3 years. EFS was 44% (95% CI 20% to 67%) at 3 years (Figure 2A). Differences in OS and EFS among the dose levels were not significant (P = 0.70 and P = 0.74, respectively).

A relapse was observed in five patients between days 54 and 265, and the cumulative incidence of relapse at 1 year was 31% (95% CI 11% to 54%; Figure 2B). Among the dose levels, no differences in the incidence of relapse were observed (two patients in the first level, one in the second and two in the third; P = 0.83, Gray's test). The cumulative incidence of TRM at 1 year was 24% (95% CI 7% to 45%).

Table 3. Toxicity by melphalan dose level

Toxicity	Patients	Grade				Toxicity events by dose level (no.)					
						Mel 130 mg		Mel 135 mg		Mel 160 mg	
		1	2	3	4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Mucositis	13	5	2	6	0	3	0	2	3	2	3
Nausea/vomit	14	3	1	10	0	2	2	1	5	1	3
Diarrhea	13	4	5	4	0	4	0	2	3	3	1
Skin rash	4	2	0	2	0	2	0	0	1	0	1
Elevated AST	14	10	4	0	0	4	0	5	0	5	0
Elevated ALP	8	8	0	0	0	1	0	4	0	3	0
Hyperbilirubinemia	8	2	5	0	1	2	0	4	0	2	1
Cardiac	2	0	1	0	1	0	1	0	0	1	0
Neurological	4	1	1	0	2	1	0	1	1	0	1
Pulmonary	1	0	0	0	1	0	0	0	0	0	1
Renal/urinary tract	3	1	0	2	0	0	0	1	1	0	1
TTP/HUS	0	0	0	0	0	0	0	0	0	0	0

Mel, melphalan; AST, serum aspartate aminotransferase; ALP, alkaline phosphatase; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome.

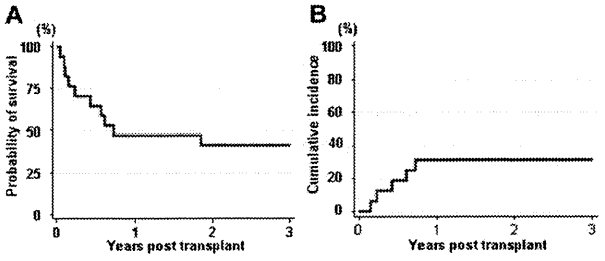

Figure 2. (A) Kaplan-Meier estimate of EFS of all assessable patients. Probability of EFS was 50% (95% CI 25% to 71%) at 1 year and 44% (95% CI 20% to 67%) at 3 years after transplant. (B) Cumulative incidence of relapse was 31% (95% CI 11% to 54%) at 1 year after transplant. EFS, event-free survival; CI, confidence interval.

cause of death. Nine of 15 patients died (Table 4). The mortality was observed in five of five in the first level, two of five in the second and two of five in the third. One patient in the first level died of chronic GVHD and another in the first level died of invasive toxoplasmosis; one patient in the second level died of secondary graft-failure. One in the first level died of the sustained disease throughout the transplantation (patient 6). A relapse was observed in five patients and was the leading cause of death in the current study.

discussion

The dosage of conditioning regimen for HSCT has not so far been determined systematically. Since we could not predict the optimal dose modification breadth and there were some promising data to show the usefulness of mCRM in dose-finding study of cancer patients [24, 25], we adopted the mCRM instead of the classical phase I approach with fixed dose levels. The stable engraftment at day 28 was defined as a primary endpoint instead of dose-limiting toxicity, which led us to the recommended dose adequately. We evaluated five patients at each dose level by using a feature of mCRM in

Table 4. Causes of death

	First level, Mel 160 mg	Second level, Mel 130 mg	Third level, Mel 135 mg	Total
Secondary graft-failure	0	1	0	1
Relapse/progression	3	1	1	5
Chronic GVHD	1	0	0	1
Infection	1	0	0	1
Hemorrhage	0	0	1	1

Mel, melphalan; GVHD, graft-versus-host disease.

which investigators could set the number of patients per dose level flexibly. One patient developed secondary graft-failure [6.3% (95% CI 0% to 30%)], a result comparable with previous reports from an unrelated BMT [26, 27]. Collectively, these results suggest that the mCRM for a conditioning regimen is a faster and safer method to determine the recommended phase II dosage.

To secure an engraftment and detect a potential effect of a modifying conditioning drug dosage on to the engraftment, we employed chimerism at a very early time point (day 28) as a primary endpoint and restricted the maximum dose modification breadth to 30 mg/m^2 to reduce the risk of graft failure. Unfortunately, we observed one secondary graft-failure even though we put a special stress on safety. However, this was the only patient mismatched in both the HLA-C and HLA-DRB1 alleles. There was also a major mismatch in ABO blood type so that the patient received the lowest number of total nuclear cells after red blood cell depletion, suggesting that both factors happened to coincide to develop this patient's secondary graft-failure [18, 28]. Although RRTs were measured by day 28 in the current study, no significant differences were observed among dose levels. In fact, we observed a very small number of toxic effects, particularly in the early time point (data not shown). Because the primary endpoint was settled as early stable chimerism, this study could be completed earlier.

Taken together, we believe that mCRM with early chimerism/engraftment as a primary endpoint is a good tool for dose-finding of HSCT conditioning.

Only one grade I acute GVHD [6% (95% CI 0% to 25%)] and no grade II–IV acute GVHD was observed in the current study, which seems considerably low, compared with other RIC-PBSCT series from HLA-matched unrelated donors [29]. This may be due to our ethnicity and/or graft source [30] or due to the strength of conditioning. As we typically observe ~35% of grade II–IV and 13% of grade III–IV acute GVHD on the basis of an HLA-match/DRB1-1 locus mismatch unrelated donor, it would be due not only to ethnicity but also to BM graft [18]. BM includes far fewer CD8+ T cells than G-PBSC, which might be attributable to the low incidence of GVHD in our patients. Since CD8+ cells in grafts have been shown to play an important role in facilitating engraftment [31–33], we should be much more careful about the conditioning dosage in BMT. Another possibility is that the strength of conditioning might be exactly adequate, leading to a modest engraftment in the current transplant settings [34]. A deeper understanding of dose–engraftment relationships in future may contribute to more stable engraftment as well as to a lower incidence of acute GVHD by RIC-BMT. On the other hand, a significant proportion of patients developed chronic GVHD [31% (95% CI 11% to 54%)], which was almost comparable with that in previous reports of conventional BMT [18, 26]. Because the incidence of chronic GVHD is a candidate surrogate for the GVL effect [5], this observation may prove beneficial for patients.

Our results verified that fludarabine plus a melphalan regimen was generally well tolerated and highly immunosuppressive. Although our follow-up is still too short to draw any conclusion about survival, given that five relapses (31%) were observed in the current study, we might consider adding some tumor-specific cytotoxic agents such as radioimmunoantibodies to reduce the likelihood of relapse [35, 36]. The OS and EFS were comparable with the previous age-matched data [37]. Further studies are warranted to confirm a long-term efficacy, particularly for lower-risk patients.

In conclusion, a phase I dose-finding study using mCRM was completed. The strategy using engraftment instead of toxicity as a primary endpoint might result in a better chance to determine the optimal dosage. Our findings have demonstrated that a melphalan dose of 135 mg/m^2 in combination with fludarabine is recommended for a further phase II evaluation.

acknowledgements

We are grateful to Seiko Amano, Mio Kurata and Asako Asano for clinical data management and data retrieval and to Fumiko Ozawa for technical assistance in chimerism analyses. This trial has been registered in the UMIN-Clinical Trial Registry System (UMIN-CTR), which is recognized as an acceptable registry by the International Committee of Medical Journal Editors. The UMIN-CTR ID for this trial is C000000325.

disclosure

The authors declare no conflicts of interest.

references

1. Childs R, Clave E, Contentin N et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 1999; 94: 3234–3241.
2. Giralt S, Estey E, Albitar M et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood 1997; 89: 4531–4536.
3. Slavin S, Nagler A, Naparstek E et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 1998; 91: 756–763.
4. McSweeney P, Niederwieser D, Shizuru J et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood 2001; 97: 3390–3400.
5. Baron F, Maris M, Sandmaier B et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol 2005; 23: 1993–2003.
6. Hassan H, Stockschläder M, Schleimer B et al. Comparison of the content and subpopulations of CD3 and CD34 positive cells in bone marrow harvests and G-CSF-mobilized peripheral blood leukapheresis products from healthy adult donors. Transpl Immunol 1996; 4: 319–323.
7. Baron F, Baker J, Storb R et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood 2004; 104: 2254–2262.
8. Corradini P, Zallio F, Mariotti J et al. Effect of age and previous autologous transplantation on nonrelapse mortality and survival in patients treated with reduced-intensity conditioning and allografting for advanced hematologic malignancies. J Clin Oncol 2005; 23: 6690–6698.
9. Goodman S, Zahurak M, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat Med 1995; 14: 1149–1161.
10. Piantadosi S, Fisher J, Grossman S. Practical implementation of a modified continual reassessment method for dose-finding trials. Cancer Chemother Pharmacol 1998; 41: 429–436.
11. Garrett-Mayer E. The continual reassessment method for dose-finding studies: a tutorial. Clin Trials 2006; 3: 57–71.
12. Lee S, Cheung YK. Model calibration in the continual reassessment method. Clin Trials 2009; 6: 227–238.
13. Wong R, Giralt S, Martin T et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood 2003; 102: 3052–3059.
14. Robak T, Korycka A, Kasznicki M et al. Purine nucleoside analogues for the treatment of hematological malignancies: pharmacology and clinical applications. Curr Cancer Drug Targets 2005; 5: 421–444.
15. Sorror M, Maris M, Storb R et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005; 106: 2912–2919.
16. Trotti A, Colevas A, Setser A et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 2003; 13: 176–181.
17. Sasazuki T, Juji T, Morishima Y et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med 1998; 339: 1177–1185.
18. Morishima Y, Sasazuki T, Inoko H et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow

transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood 2002; 99: 4200–4206.
19. Przepiorka D, Khouri I, Ippoliti C et al. Tacrolimus and minidose methotrexate for prevention of acute graft-versus-host disease after HLA-mismatched marrow or blood stem cell transplantation. Bone Marrow Transplant 1999; 24: 763–768.
20. Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995; 15: 825–828.
21. Sullivan K, Agura E, Anasetti C et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol 1991; 28: 250–259.
22. Ohashi H, Kato C, Fukami S et al. Leukemic relapse in the central nervous system after allogeneic stem cell transplantation with complete remission in the bone marrow and donor-type chimerism: report of two cases. Am J Hematol 2005; 79: 142–146.
23. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457–481.
24. Morita S, Nakata B, Tsuji A et al. A phase I study of combination therapy of the oral fluorinated pyrimidine compound S-1 with low-dose cisplatin twice-a-week administration (JFMC27-9902 Step2) in patients with advanced gastric cancer using a continual reassessment method. Jpn J Clin Oncol 2007; 37: 924–929.
25. Loeb D, Garrett-Mayer E, Hobbs R et al. Dose-finding study of 153Sm-EDTMP in patients with poor-prognosis osteosarcoma. Cancer 2009; 115: 2514–2522.
26. Kodera Y, Morishima Y, Kato S et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. Bone Marrow Transplant 1999; 24: 995–1003.
27. Kusumi E, Kami M, Yuji K et al. Feasibility of reduced intensity hematopoietic stem cell transplantation from an HLA-matched unrelated donor. Bone Marrow Transplant 2004; 33: 697–702.
28. Mattsson J, Ringdén O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2008; 14: 165–170.
29. Giralt S, Logan B, Rizzo D et al. Reduced-intensity conditioning for unrelated donor progenitor cell transplantation: long-term follow-up of the first 285 reported to the national marrow donor program. Biol Blood Marrow Transplant 2007; 13: 844–852.
30. Oh H, Loberiza FJ, Zhang M et al. Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. Blood 2005; 105: 1408–1416.
31. Martin P. Donor CD8 cells prevent allogeneic marrow graft rejection in mice: potential implications for marrow transplantation in humans. J Exp Med 1993; 178: 703–712.
32. Martin P, Rowley S, Anasetti C et al. A phase I–II clinical trial to evaluate removal of CD4 cells and partial depletion of CD8 cells from donor marrow for HLA-mismatched unrelated recipients. Blood 1999; 94: 2192–2199.
33. Martin P, Hansen J, Buckner C et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood 1985; 66: 664–672.
34. Turner B, Kambouris M, Sinfield L et al. Reduced intensity conditioning for allogeneic hematopoietic stem-cell transplant determines the kinetics of acute graft-versus-host disease. Transplantation 2008; 86: 968–976.
35. Pagel J, Gooley T, Rajendran J et al. Allogeneic hematopoietic cell transplantation after conditioning with 131I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. Blood 2009; 114: 5444–5453.
36. Pagel J, Orgun N, Hamlin D et al. A comparative analysis of conventional and pretargeted radioimmunotherapy of B-cell lymphomas by targeting CD20, CD22, and HLA-DR singly and in combinations. Blood 2009; 113: 4903–4913.
37. Kojima R, Kami M, Kanda Y et al. Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years. Bone Marrow Transplant 2005; 36: 667–674.

ASBMT
American Society for Blood and Marrow Transplantation

Comparison of Unrelated Cord Blood Transplantation and HLA-Mismatched Unrelated Bone Marrow Transplantation for Adults with Leukemia

Yoshiko Atsuta,[1] Yasuo Morishima,[2,] Ritsuro Suzuki,[1] Tokiko Nagamura-Inoue,[3] Shuichi Taniguchi,[4] Satoshi Takahashi,[5] Shunro Kai,[6] Hisashi Sakamaki,[7] Yasushi Kouzai,[8] Naoki Kobayashi,[9] Takahiro Fukuda,[10] Hiroshi Azuma,[11] Minoko Takanashi,[12] Takehiko Mori,[13] Masahiro Tsuchida,[14] Takakazu Kawase,[15] Keisei Kawa,[16] Yoshihisa Kodera,[17] Shunichi Kato,[18,*] for the Japan Marrow Donor Program and the Japan Cord Blood Bank Network*

Recent advances in unrelated cord blood transplantation (UCBT) and high-resolution typing of human leukocyte antigen (HLA) from an unrelated donor have increased choices in alternative donor/stem cell source selection. We assessed HLA-mismatched locus-specific comparison of the outcomes of 351 single-unit UCB and 1,028 unrelated bone marrow (UBM) adult recipients 16 years old or older at the time of transplantation who received first stem cell transplantation with myeloablative conditioning for acute leukemia or myelodysplastic syndromes. With adjusted analyses, HLA 0 to 2 mismatched UCBT showed similar overall mortality (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; $P = .149$) compared with that of single-HLA-DRB1-mismatched UBMT. UCBT showed inferior neutrophil recovery (RR = 0.50, 95% CI, 0.42-0.60; $P < .001$), lower risk of acute graft-versus-host disease (RR = 0.55, 95% CI, 0.42-0.72; $P < .001$), and lower risk of transplantation-related mortality (RR = 0.68, 95% CI, 0.50-0.92; $P = .011$) compared with single-HLA-DRB1-mismatched UBMT. No significant difference was observed for risk of relapse (RR = 1.28, 95% CI, 0.93-1.76; $P = .125$). HLA 0 to 2 antigen-mismatched UCBT is a reasonable second alternative donor/stem cell source with a survival outcome similar to that of single-HLA-DRB1-mismatched or other 7 of 8 UBMT.

Biol Blood Marrow Transplant 18: 780-787 (2012) © *2012 American Society for Blood and Marrow Transplantation*

KEY WORDS: Unrelated cord blood transplantation, HLA-mismatched unrelated bone marrow transplantation

From the [1]Department of HSCT Data Management/Biostatistics Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Hematology and Cell Therapy Aichi Cancer Center Hospital, Nagoya, Japan; [3]Department of Cell Processing & Transfusion, Research Hospital The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank Tokyo, Tokyo, Japan; [4]Department of Hematology Toranomon Hospital, Tokyo, Japan; [5]Department of Molecular Therapy The Institute of Medical Science The University of Tokyo, Tokyo, Japan; [6]Department of Transfusion Medicine Hyogo College of Medicine, Nishinomiya, Japan; [7]Division of Hematology Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [8]Department of Transfusion Medicine, Tokyo Metropolitan Tama Medical Center, Tokyo, Japan; [9]Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan; [10]Hematopoietic Stem Cell Transplantation Unit National Cancer Center Hospital, Tokyo, Japan; [11]Hokkaido Red Cross Blood Center, Sapporo, Japan; [12]The Japanese Red Cross Tokyo Blood Center, Tokyo, Japan; [13]Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan; [14]Ibaraki Children's Hospital, Mito, Japan; [15]Division of Epidemiology and Prevention, Aichi Cancer Center Hospital, Nagoya, Japan; [16]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; [17]BMT Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; and [18]Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan.

Financial disclosure: See Acknowledgments on page 786.

*Y.M. and S. Kato share senior authorship.

Correspondence and reprint requests: Yoshiko Atsuta, MD, PhD, Department of Hematopoietic Stem Cell Transplantation Data Management/Biostatistics, Nagoya University School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku Nagoya 461-0047, Japan (e-mail: y-atsuta@med.nagoya-u.ac.jp).

Received July 20, 2011; accepted October 9, 2011

1083-8791/$36.00
doi:10.1016/j.bbmt.2011.10.008

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a widely used, curative treatment for hematologic malignancies. When available, a human leukocyte antigen (HLA)-identical sibling is the donor of choice. However, only about 30% of candidates eligible for allogeneic HSCT will have such a donor. In addition, older patients with older siblings have more difficulty finding such a donor capable of stem cell donation. High-resolution donor-recipient HLA matching has contributed to the success of unrelated donor marrow transplantation, and the current first recommended alternative donor after an HLA-matched sibling for HSCT is an HLA-A, -B, -C, and -DRB1 8 of 8-allele-matched unrelated donor [1-4]. However, there are still a significant number of patients for which finding an HLA 8 of 8-matched unrelated donor is difficult and for whom a second alternative donor/stem cell source should be found.

The effect of HLA mismatches after bone marrow transplantation from unrelated donors (UBMT) has been well studied, and single mismatched UBM donors are usually selected as a second alternative donor/stem cell source [1-4]. Lee al. [3] showed that a single mismatch, antigen-level, or high-resolution, at HLA-A, -B, -C, or -DRB1 loci was associated with higher mortality and decreased survival. However, the reduction in survival may be acceptable in comparison with the survival rates for currently available alternative treatments. Analyses from the Japan Marrow Donor Program (JMDP) showed better survival in HLA class II mismatched recipients; thus, single-DRB1-mismatched UBM donor is currently a second alternative in Japan [1,2,5].

Recent advances in unrelated cord blood transplantation (UCBT) have provided patients with increased choices for a second alternative donor/stem cell source [6]. Clinical comparison studies of cord blood transplantation and HLA-A, -B, and -DRB1 6 of 6 allele-matched bone marrow transplantation for leukemia from unrelated donors in adult recipients showed comparable results [7-9]. More recently, promising outcomes of UCBT were shown compared with HLA-A, -B, -C, and -DRB1 8 of 8 allele-matched UBMT, the current first alternative donor/stem cell source [10-12].

The aim of this study was to determine the utility of UCBT as a second-alternative donor source in adult patients with acute leukemia or myelodysplastic syndromes. It is common today to perform high-resolution typing of HLA for donor selection of unrelated donors; thus, we performed mismatched-allele-specific analyses for comparison of HLA-mismatched UBMT and UCBT in terms of overall survival (OS) and other HSCT outcomes, setting single-DRB1-mismatched UBMT, the current second alternative, as the reference.

PATIENTS AND METHODS

Collection of Data and Data Source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the JMDP [13]. Peripheral blood stem cell donation from unrelated donors was not permitted in Japan during the study period. All 11 cord blood banks in Japan are affiliated with JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days posttransplantation. Patients' information on survival, disease status, and long-term complications including chronic graft-versus-host (cGVHD) disease and second malignancies is renewed annually using follow-up forms. This study was approved by the institutional review board of Nagoya University Graduate School of Medicine.

Patients

The subjects were adult patients of at least 16 years of age with acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndromes, who were recipients of first UBMT or UCBT with myeloablative conditioning. All patients in the UCBT cohort received a single-unit CB. Transplantation years were between 1996 and 2005 for UBMT and between 2000 and 2005 for UCBT to avoid the first 3 years of a pioneering period (1993-1995 for UBMT and 1997-1999 for UCBT). There were no statistically significant differences between UBMT in 1996-1999 and UBMT in 2000-2005 in probabilities of OS (41% versus 44%, at 3 years posttransplantation; $P = .86$) and in relapse-free survival (RFS) (40% versus 40%, at 3 years posttransplantation; $P = .93$).

Among 2,253 UBMT recipients with complete HLA high-resolution data, the following recipients with HLA -A, -B, -C, and -DRB1 8 of 8 allele match (n = 1,079) and more than three mismatches (5 of 8 allele match [n = 117], 4 of 8 allele match [n = 24], 3 of 8 allele match [n = 4], 2 of 8 allele match [n = 1]) were excluded. There were no statistically significant differences in risk of mortality or treatment failure (RFS) associated with single high-resolution (allele) versus single low-resolution (antigen) mismatches (data not shown), so in the analyses, allele and antigen mismatches were considered equivalent. HLA matching of cord blood was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 557 recipients of CB with complete HLA data, 105 recipients with three mismatches and nine recipients with four mismatches were excluded. A total of 1,028 UBMT recipients (248 HLA class II locus mismatched, 424 HLA class I locus mismatched, and 356 HLA 2 loci mismatched) and 351 UCBT recipients (20 HLA-A, -B, low-resolution and -DRB1 matched, 87

locus mismatched, and 244 2 loci mismatched) were the subjects for analyses. Both host-versus-graft and graft-versus-host directions were accounted for in terms of HLA mismatch.

HLA Typing

Alleles at the HLA-A, -B, -C, and -DRB1 with unrelated bone marrow donor-recipient pairs and for HLA-DRB1 for unrelated cord blood donor-recipient pairs were identified by the methods described previously [1,5,14]. Serologic or antigen-level typing was performed with a standard two-stage complement-dependent test of microcytotoxicity or low-resolution DNA-based typing usually by collapsing the four-digit typing result back to its first two digits in part.

Definitions

The primary outcome of the analyses was OS, defined as time from transplantation to death from any cause. A number of secondary endpoints were also analyzed. Neutrophil recovery was defined by an absolute neutrophil count of at least 500 cells per cubic millimeter for three consecutive points; platelet recovery was defined by a count of at least 50,000 platelets per cubic millimeter without transfusion support. Diagnosis and clinical grading of acute GVHD (aGVHD) were performed according to the established criteria [15,16]. Relapse was defined as a recurrence of underlying hematologic malignant diseases. Transplantation-related death was defined as death during a continuous remission. RFS was defined as survival in a state of continuous remission.

Statistical Analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. Medians and ranges are provided for continuous variables and percentages for categoric variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of aGVHD and cGHVD, relapse, and transplantation-related mortality (TRM) [17]. Gray's test was used for group comparison of cumulative incidences [18]. Adjusted comparison of the groups on OS and RFS was performed with the use of the Cox proportional-hazards regression model [19]. For other outcomes with competing risks, Fine and Gray's proportional-hazards model for subdistribution of a competing risk was used [20]. For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and, for TRM, relapse was the competing event [21]. Adjusted probabilities of OS and RFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were the patient's age at transplantation, patient's sex, donor-patient sex mismatch, donor-patient ABO mismatch, diagnosis, disease status at conditioning, the conditioning regimen, and the type of prophylaxis against GVHD. Factors differing in distribution between CB and BM recipients and factors known to influence outcomes were included in the final models. Variables with more than two categories were dichotomized for the final multivariate model. Variables were dichotomized as follows: patient age >40 or <40 years at transplantation, recipient's sex, sex-mismatched donor-patient pair versus sex-matched pair, donor-recipient ABO major mismatch versus others for ABO matching, advanced versus standard (first and second complete remission of acute myeloid leukemia, first complete remission of acute lymphoblastic leukemia, or refractory anemia or refractory anemia with ring sidoblasts of myelodysplastic syndromes) risk of the disease, cyclophosphamide, and total-body irradiation (TBI) or busulfan and cyclophosphamide or others for conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against GVHD. No significant interactions were identified between each variable and HLA disparity/stem cell source groups. All P values were two-sided.

RESULTS

Patient Characteristics

Table 1 shows characteristics of patients, their disease, and transplantation regimens. Proportions of females, sex-mismatched donor-recipient pairs, and ABO mismatched donor recipient pairs were larger in cord blood recipients ($P < .001$, $P < .001$, and $P < .001$, respectively). UCB recipients were older than recipients of UBM (median age, 37 years versus 34 years; $P < .001$). A preparative regimen with TBI and cyclophosphamide was used in the majority of patients in all groups, and cytosine arabinoside was supplemented for CB recipients in addition to TBI and cyclophosphamide in about half the recipients with cyclophosphamide and TBI. For GVHD prophylaxis, tacrolimus and short-term methotrexate was used preferentially in BM recipients (61% of DRB1-one-mismatched BM recipients), while cyclosporine A and short-term methotrexate was used preferentially in CB recipients (61%). The median follow-up period for survivors was 2.1 years (range, 0.1-6.2) for CB recipients and 5.5 (range, 0.3-11.6) years for BM recipients.