multiple mechanisms, with some being BCR-ABL dependent and others BCR-ABL independent. To overcome the failure of imatinib, multiple strategies are under investigation. These strategies include a dose escalation of imatinib and switching to second-generation TKIs. Nilotinib and dasatinib are currently approved for the treatment of patients with CML who have developed resistance or intolerance to imatinib [5, 6].

The development of a T315I BCR-ABL mutation (threonine to isoleucine mutation at amino acid 315) is of particular concern as it confers resistance to all available TKIs [7–10]. The only established salvage option for patients harboring the T315I BCR-ABL mutation is allogeneic hematopoietic stem cell transplantation (allo-HSCT) [11–13]. However, allo-HSCT can be performed only in eligible patients [14]. For patients who could not receive allo-HSCT, new agents with activity against the T315I BCR-ABL mutation, such as danusertib and omacetaxin, have been developed [15, 16]. However, they are still in the clinical trial stage and it will take years before these agents can be put into use. Hence, patients harboring the T315I BCR-ABL mutation, who are not eligible for allo-HSCT, require treatment with combinations of already approved drugs.

We report the successful treatment of a CML patient harboring the T315I BCR-ABL mutation with a combination of imatinib and IFNα.

Materials and methods

Total RNA extraction and cDNA synthesis

Total leukocytes in bone marrow and peripheral blood samples were isolated by centrifugation following red blood cell lysis and total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). cDNA was synthesized using oligo-dT primers and Super Script III Reverse Transcriptase (Invitrogen).

TaqMan quantitative reverse transcriptase-polymerase chain reaction

Quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) for BCR-ABL transcript levels were performed using the LightCycler (Roche Diagnostics, Mannheim, Germany) and LightCycler TaqMan Master (Roche Diagnostics). Primers and TaqMan probe sequences published in the EAC network protocol were used for RQ-PCR [17]. The amount of the fusion gene in the original sample was calculated by means of a standard curve (created with the BCR-ABL fusion gene or the ABL gene cloned in plasmids) and expressed as the BCR-ABL/ABL ratio.

Direct sequencing of ABL kinase domain

A nested PCR sequencing approach was used for direct sequencing of the ABL kinase domain, with a first-round amplification of the BCR-ABL transcript followed by two separate PCR reactions. For the nested PCR, the primers were used as described previously [18, 19]. To screen for mutations, the PCR products were sequenced in both the directions with the following: ABL-1F (5′-ACAGGATCAACACTGCT TCTGA-3′); ABL-1R (5′-TGGCTGACGAGATCTGAGTG-3′); ABL-2F (5′-ATGGCCACTCAGATCTCGTC-3′); and ABL-2R (5′-GATACTGGATTCCTGGAACA-3′) using a BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3100xl Genetic Analyzer (Applied Biosystems, CA, USA).

Quantitative T315I BCR-ABL mutational analysis by pyrosequencing

Quantitation of T315I BCR-ABL and un-mutated BCR-ABL transcript levels were performed using the PyroMark ID Pyrosequencing system (QIAGEN). First-round PCR was carried out followed by second-round PCR for T315I BCR-ABL mutation including one biotin-labeled primer. Primers and PCR conditions were used as described previously [20]. The linearity of quantitative T315I BCR-ABL mutation by pyrosequencing was confirmed by subjecting cDNA generated from graded mixes of Ba/F3 cell lines (RIKEN Cell Bank, Tsukuba, Japan) transfected with BCR-ABL cDNAs containing either the un-mutated BCR-ABL sequence or the T315I BCR-ABL mutation.

Case report

A 61-year-old male was referred to our hospital due to leukocytosis, thrombocytosis, and hepatosplenomegaly (hypochondrial spleen size 8 cm) in October 2002. Complete blood cell analysis showed that the white blood cell count was 138,900/μl, with 36% neutrophils, 3% myeloblasts, 5% promyelocytes, 5% myelocytes, 14% metamyelocytes, 6% lymphocytes, 5% monocytes, 5% basophils, and 3% eosinophils; hemoglobin concentration was 11.2 g/dl; and the platelet count was 122.1 × 10^4/μl. Bone marrow analysis showed hypercellularity with significant myeloid hyperplasia with 3.0% myeloblasts. Chromosomal analysis (G-banding) revealed that there were no additional chromosomal abnormalities other than t(9;22)(q34;q11). No BCR-ABL kinase domain mutation was detected by direct sequencing (Fig. 1a) and also by pyrosequencing. He was diagnosed with CP-CML. The Sokal score was 1.94, indicating high risk.

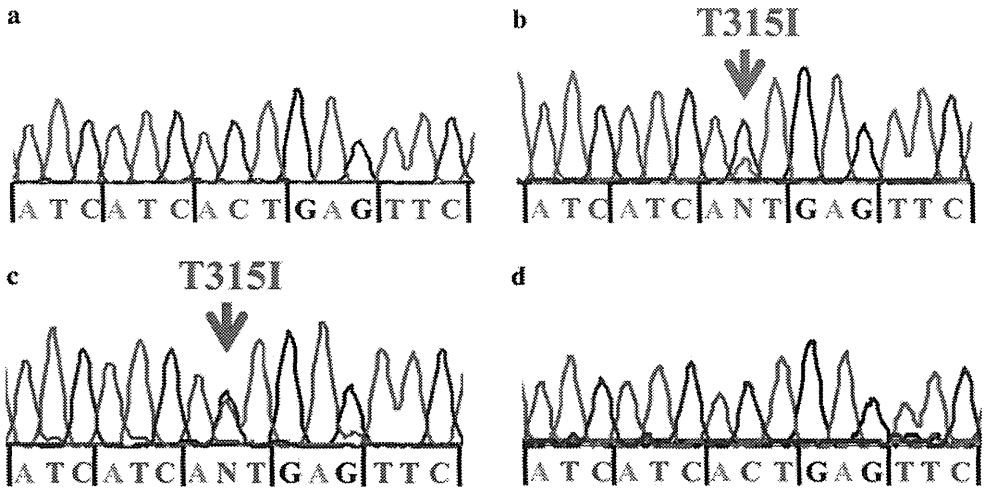

Fig. 1 T315I BCR-ABL mutation by direct sequencing: **a** at diagnosis, **b** at 18 months after starting imatinib, **c** at 24 months after starting imatinib, **d** at 51 months after starting the combination therapy

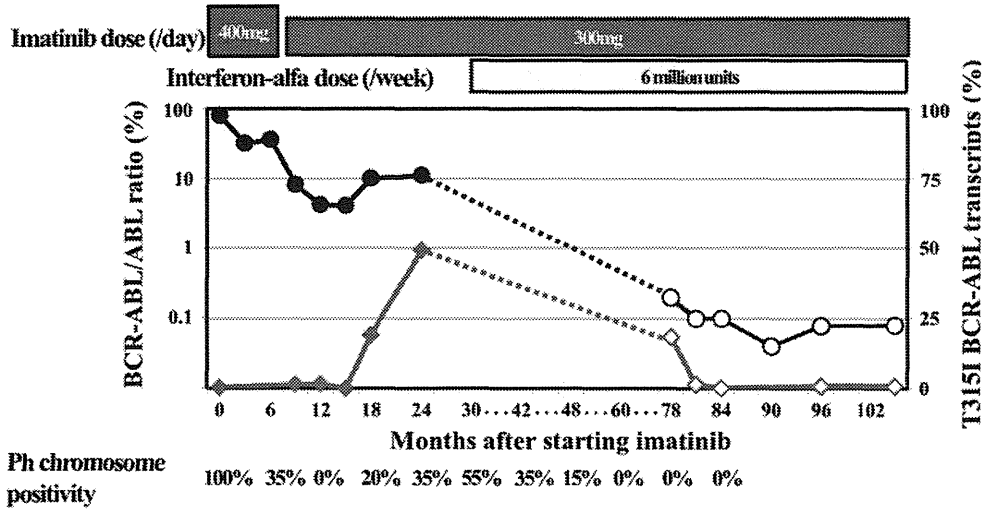

Fig. 2 Clinical course of total and T315I BCR-ABL mutant transcript levels. The figure shows total BCR-ABL transcript levels (*solid line*) measured by RQ-PCR and the relative size of T315I BCR-ABL mutant transcript levels (*dotted line*) by pyrosequencing. The *filled circle* and *filled square* represent samples from bone marrow, and the *open circle* and *open square* represent samples from peripheral blood. Ph chromosome positivity (%) represents the ratio of Ph-positive cells in bone marrow cells determined by G-band chromosomal analysis

He was registered in the clinical trial (Japan Adult Leukemia Study Group, CML202 study) and imatinib was initiated with a dose of 400 mg/day in October 2002. A dose reduction (300 mg/day) was necessary after 6 months due to muscle cramp, which was considered to be a side effect. Complete hematologic response (CHR) and complete cytogenetic response (CCyR) were achieved within 1 and 12 months of treatment, respectively. However, after 18 months of imatinib treatment, a loss of CCyR was observed and a direct sequencing study at 24 months revealed a T315I mutation of the BCR-ABL gene (Fig. 1b). The earlier samples (at 18 months) were then analyzed retrospectively and the mutation was also identified. Even though pyrosequencing revealed that T315I transcripts increased over 2.5-fold during the 18- to 24-month period (Fig. 1c), total BCR-ABL transcripts measured by a RQ-PCR remained unchanged: ratios of BCR-ABL to ABL were 10.1% at 18 months and 11.1% at 24 months, respectively. Because a loss of the major cytogenetic response occurred at 30 months, a combination therapy which consisted of imatinib and IFNα was initiated. IFNα was administrated at a dose of 6 million Units/week. Thirty months after the initiation of the imatinib/IFNα combination therapy, he re-achieved CCyR. Forty-eight months after, the T315I BCR-ABL mutation remained detectable although CCyR was maintained. After 51 months, RQ-PCR revealed a reduction of BCR-ABL transcripts by 3 or more logs [i.e., major molecular response (MMR)], and the T315I BCR-ABL mutation was not detected by direct sequencing and pyrosequencing (Fig. 1d). The MMR was still maintained at 75 months after the initiation of the imatinib/IFNα combination therapy without any signs of a recurrence of the T315I BCR-ABL mutation (Fig. 2). Although he experienced grade 2 anemia, grade 1 neutropenia, and thrombocytopenia according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0, it was possible to continue the imatinib/IFNα combination therapy with no dose reduction.

Discussion

The current treatment algorithm for patients with CML suggests that if the patient develops a T315I BCR-ABL mutation, allo-HSCT or participation in clinical trials should be considered (new agents against the T315I BCR-ABL mutation [15, 16, 21–24] are still in trials). In our case, the imatinib/IFNα combination therapy used resulted in MMR, suggesting its effectiveness in patients harboring the T315I BCR-ABL mutation. De Lavallade et al. [25] have reported the clinical outcome for a CML patient who acquired the T315 BCR-ABL mutation while on imatinib, that was treated successfully with IFNα alone. In their report, while the level of T315I BCR-ABL mutant transcripts decreased with the interferon therapy, the total amount of BCR-ABL transcripts was relatively stable, suggesting that the CML clone harboring an un-mutated BCR-ABL was expanding during that period. To prevent this phenomenon, we chose a combination therapy with imatinib and IFNα. This therapy theoretically seemed reasonable because it would inhibit both the T315I-mutated and the un-mutated BCR-ABL clone, and as shown in this report, it was quite successful. Determining whether or not the T315I BCR-ABL mutated clone is more susceptible to IFNα than an un-mutated clone would be of interest.

In conclusion, although our experience is limited to one patient, imatinib/IFNα combination therapy could be a viable treatment option for CP-CML patients with a T315I BCR-ABL mutation. Further studies are necessary to confirm the efficacy and applicability of imatinib/IFNα combination therapy.

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ORIGINAL ARTICLE

Randomized comparison of fixed-schedule versus response-oriented individualized induction therapy and use of ubenimex during and after consolidation therapy for elderly patients with acute myeloid leukemia: the JALSG GML200 Study

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Abstract We conducted a multicenter prospective randomized study to compare a fixed-scheduled induction therapy with a response-oriented individualized induction therapy for elderly patients with acute myeloid leukemia (AML). Newly diagnosed AML patients, aged between 65 and 80, were randomly assigned to receive fixed or individualized induction. Both groups received daunorubicin (DNR) 40 mg/m^2 for 3 days and behenoyl cytarabine (BHAC) 200 mg/m^2 for 8 days. In the individualized group, bone marrow biopsy was done on days 8 and 10, and according to the cellularity and blast ratio, the patients received additional DNR and BHAC for two to four more days. All patients achieving complete remission (CR) were randomized a second time to determine whether they would receive ubenimex. CR was obtained in 60.1 % of the fixed group and 63.6 % of the individualized group.

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Predicted 4-year relapse-free survival (RFS) was 9 % for the fixed group and 18 % for the individualized group. There were no statistically significant differences in CR and RFS between the fixed and individualized groups. In the ubenimex group, prolonged RFS was observed. Notably, gender was a prognostic factor in this study, as 102 female patients had a significantly higher CR rate (72.5 vs. 54.3 %, $p = 0.0048$) and better OS (24 vs. 14 % at 4 years, $p = 0.018$), compared with 140 male patients.

Keywords Acute myeloid leukemia · Elderly · Response-oriented individualized induction therapy · Daunorubicin · Behenoyl cytarabine (enocitabine, BHAC)

Introduction

With the extension of life-span, elderly patients with acute myeloid leukemia (AML) are increasing in number, and the median age of AML is presently around 65–70. Prognosis of these patients is poorer, compared with younger patients, as their complete remission (CR) rate is around 50 % and overall survival (OS) is <20 % at 5 years, showing no remarkable progress during the past decades, despite every possible effort by many investigators. Regrettably, there is no recommendable standard regimen effective enough for the treatment of elderly AML [1–6].

In Japan, a response-oriented individualized induction therapy has been employed for AML since the DCMP two-step therapy, using daunorubicin (DNR), cytarabine (Ara-C), 6-mercaptopurine (6MP) and prednisolone (PSL) by Uzuka et al. in the mid 1970s, reporting more than 80 % CR rate, which is not surprisingly high today but was remarkable at that time even for a single institutional study [7]. Subsequently, a response-oriented individualized BHAC-DMP induction therapy, using behenoyl Ara-C (BHAC, enocitabine), DNR, 6MP and PSL, was developed by Ohno et al. [8], reporting more than 80 % CR in adult AML by a single institutional study. A multi-institutional AML87 study, conducted by the Japan Adult Leukemia Study Group (JALSG), confirmed the high CR rate of BHAC-DMP therapy for adult AML, resulting in 80 % CR rate [9]. Succeeding JALSG studies, AML89 [10] and AML92 [11] also employed the response-oriented individualized induction therapy and reported 81 and 77 % CR rates, respectively, for younger adult patients with non-M3 type AML. These CR rates were around 10 % higher than those reported from cooperative study groups in the USA and Europe, where fixed-scheduled induction therapies were employed [3, 12–14].

However, after clinical introduction of idarubicin (IDR), a more potent derivative of DNR, the JALSG AML95 study which prospectively compared the two treatment schedules, using Ara-C and IDR instead of DNR, could not demonstrate any advantage of the response-oriented individualized induction therapy over the fixed-scheduled induction therapy for younger patients with AML of age <65 [15].

In the present study, with elderly AML patients of age from 65 to 80, we compared a response-oriented individualized induction therapy with a fixed-scheduled induction therapy using BHAC and DNR. Additionally, we randomly compared the effectiveness of ubenimex among patients who had achieved CR by these two induction regimens. Ubenimex, a dipeptide immunostimulator, reportedly prolonged OS and disease-free survival in adult AML patients when used during and after consolidation therapy [16–18].

Materials and methods

Patients

From August 2000 to December 2005, all newly diagnosed elderly patients with AML were consecutively registered from 55 institutions which participated in this study. Informed consent was obtained from all the patients before registration in accordance with the Declaration of Helsinki. AML was first diagnosed by the French–American–British (FAB) classification at each institution. Peripheral blood and bone marrow smears from all registered patients were sent to Nagasaki University, and examined with May-Giemsa, peroxidase and esterase staining. Then, diagnosis was reevaluated by the central review committee. Eligibility criteria for the randomization study included age from 65 to 80 years, AML by FAB classification except M3, adequate functioning of the liver (serum bilirubin level <2.0 mg/dL), kidney (serum creatinine <2.0 mg/dL), heart (ejection fraction >50 %) and lungs, an Eastern Cooperative Oncology Group performance status between 0 and 2,

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and written informed consent for the randomized study. Patients were not eligible if they had pre-diagnosed myelodysplastic syndromes (MDS), but were eligible if they had no definite diagnosis of MDS, even when they had previous history of hematological abnormality. Patients with ill-controlled diabetes mellitus, angina pectoris, infectious episodes and liver cirrhosis were not eligible, as well as those with positive HIV antibody, HCV antibody and HB antigen. Patients who did not meet the eligibility criteria or did not agree to the randomization study were included also for the initial evaluation and survival. Cytogenetic analysis was performed by standard methods of G-banding, and abnormalities were grouped according to the MRC classification [19]. The protocol was approved by the institutional review board of each hospital.

Treatment regimens

Eligible patients who had given their informed consent for the randomized study were assigned to receive either a fixed-scheduled induction therapy or a response-oriented individualized induction therapy through a centralized computer system. All assigned patients received DNR 40 mg/m^2/day by 30-min infusion on days 1–3 and BHAC 200 mg/m^2/day by 3-h infusion on days 1–8. For patients of age 70 or older, the dose of DNR was reduced to 30 mg/m^2/day. In the individualized group, bone marrow aspiration was performed on day 8, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 9 and 10. If 20–50 % of blasts remained, DNR was added on day 8, and if more than 50 % of blasts remained, DNR was added on days 8 and 9. Another bone marrow aspiration was performed on day 10, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 11 and 12. If 20–50 % of blasts remained, DNR was added on day 11, and if more than 50 % of blasts remained, DNR was added on days 11 and 12 (Fig. 1). If patients had documented infection or other complications on day 8 or day 11, cancellation of additional chemotherapy was permitted by the attending physician's judgment. In the fixed-scheduled group, patients did not receive additional doses, regardless of their marrow status at day 8. If patients did not achieve CR by the first course, the same induction therapy was repeated at approximately 3- to 4-week interval. If patients did not achieve CR with two courses, these cases were judged as failure.

All patients who had achieved CR received 3 courses of consolidation therapy, and were randomly assigned either to receive daily 30 mg of ubenimex (Bestatin, Nippon Kayaku, Tokyo, Japan) or not, concomitantly during the consolidation therapy. The first course of consolidation consisted of BHAC (200 mg/m^2 by 3-h infusion on days 1–5) and mitoxantrone (MIT, 7 mg/m^2 by 30-min infusion on days 1–3). The second consisted of BHAC (200 mg/m^2 on days 1–7), DNR (30 mg/m^2 by 30-min infusion on days 1–2) and etoposide (ETP; 100 mg/m^2 by 1-h infusion on days 1–3). The third consisted of BHAC (200 mg/m^2 on

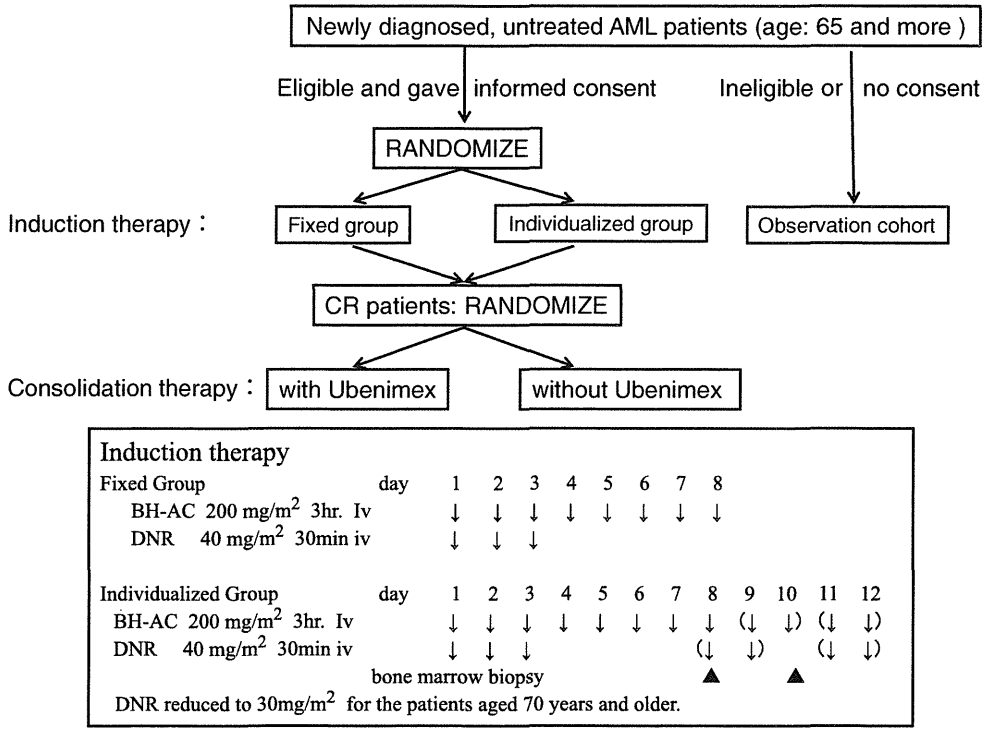

Fig. 1 Consort diagram and treatment schedule of induction therapy. Eligible patients were randomized to fixed group or individualized group. Patients achieved complete remission were done second randomization to with ubenimex or without ubenimex. Induction therapy in individualized group, BHAC dosage should be escalated up to twelve doses and up to seven doses for daunorubicin according to the bone marrow state

days 1–5) and aclarubicin (ACR; 14 mg/m^2 by 30-min infusion on days 1–5). For patients of age 70 or more, the dose of MIT, DNR, ETP and ACR was reduced to 5, 25, 75 and 10 mg/m^2, respectively. Each consolidation course was given as soon as possible after the leukocyte and platelet counts had recovered to more than 3,000 and 100,000/μL, respectively. Intrathecal methotrexate (15 mg), Ara-C (40 mg) and PSL (10 mg) were given after the third consolidation therapy for the prophylaxis of central nervous system leukemia. Patients assigned to be given ubenimex received it for 3 more months after the completion of consolidation therapy, but no further chemotherapy was given to either group. For non-eligible patients or for those who did not give informed consent for the randomized study, no intervention was specified and the therapy was left to the decision of attending physicians. However, their OS data were reported.

Best supportive care, including administration of antibiotics and platelet transfusion from blood cell separators, was given if indicated. When patients had life-threatening infections during neutropenia, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

Response criteria and statistical analysis

CR was defined as the presence of all the following criteria: <5 % of blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts over 1,000/μL and platelet counts over 100,000/μL, and no evidence of extramedullary leukemia. CR had to continue for at least 4 weeks, but the date of CR was defined as the first day when these criteria were fulfilled. Relapse was defined as the presence of at least one of the following: recurrence of more than 10 % leukemic cells in bone marrow, any leukemic cells in peripheral blood, and appearance of extramedullary leukemia.

Overall survival (OS) was calculated from the diagnostic day to death by any cause, and censored at the last follow-up. Relapse-free survival (RFS) for patients who achieved CR was measured from the date of CR to relapse or death by any cause, and censored at the last follow-up.

This was a multi-institutional randomized phase 3 study with a 2 × 2 factorial design. The primary end point of the first randomization was CR rate, and the secondary endpoints were OS and RFS. For the second randomization, the primary end point was RFS and the secondary endpoint was OS, and Kaplan–Meier product limit estimation was used to determine OS and RFS. A sample size of 98 patients per group was estimated to have a power of 70 % at a 5 % level of significance (single-sided) to demonstrate 10 % non-inferiority in CR rate (60 vs. 55 %). Statistical testing for the non-inferior trial was performed according to the method of Blackwelder [20]. To test the factors to predict CR, χ^2 test and Wilcoxon rank-sum test were used for univariate analysis, and the multiple logistic regression model was used for multivariate analysis. For comparison of OS and RFS, the log-rank test and the generalized Wilcoxon test were used for univariate analysis and Cox's proportional hazard model was used for multivariate analysis. SAS ver. 8.2 (SAS Institute Inc., Cary, NC, USA) was used for the analysis. p values <0.05 (two-sided) were considered statistically significant. Analysis was done on an intent-to-treat basis. This study is registered at http://www.umin.ac.jp/ctrj/ as C000000220 for the randomization study on eligible patients and C000000224 for the observation study on non-eligible patients.

Results

Patient population and characteristics

Of 375 patients registered, 130 patients were either judged as non-eligible by the attending physicians because of various reasons listed in eligibility criteria, including 6 patients with FAB-M3, or eligible but gave no informed consent to enter the randomized study. Of 245 eligible and consented patients, 122 were assigned to the fixed-scheduled therapy and 123 to the individualized therapy. One in the former group and two in the latter were unevaluable due to insufficient data. Pretreatment characteristics of 242 evaluable patients are presented in Table 1. Overall, the median age was 71, and 47 patients (19 %) were of age 75 or older. Successful cytogenetic data were reported in 231 patients (95 %), including 113 patients (91 %) in 124 observation cohort excluding M3. There were no major imbalances between the two randomized groups, although there were fewer patients with favorable cytogenetics and more with adverse cytogenetics in the fixed-scheduled group ($p = 0.1338$) (Table 1).

In the individualized therapy group, during the first course of the induction therapy, 45 patients received additional doses of DNR and BHAC from day 9, and 13 patients received the additional doses from day 11, and, during the second course, 11 patients received additional doses from day 9 and 2 from day 11.

Overall treatment results

Of 242 evaluable patients, 150 (62.0 %) achieved CR. Of 121 patients in the fixed-scheduled group, 73 (60.3 %) obtained CR, and of 121 in the individualized group 77 (63.6 %) achieved CR ($p = 0.6913$). In the fixed-scheduled group, 56 patients (46.3 %) achieved CR after the first course, while in the individualized group 56 patients (46.3 %) achieved CR after the first course. Of 53 (43.8 %)

Table 1 Patient characteristics

	Fixed-scheduled	Individualized	Non-randomized	Total
No. of patients	121	121	124	366
Age (years)				
65–69	54	51	29	134
70–74	42	48	36	126
75–79	25	22	32	79
80–	0	0	27	27
Median (range)	70 (65–79)	71 (65–79)	74 (65–92)	
Chromosome				
Favorable	6	14	7	27
Intermediate	91	92	91	274
Adverse	18	10	15	43
Unknown	6	5	11	22
FAB classification				
M0	10	8	10	28
M1	24	23	32	79
M2	48	52	45	145
M4	18	18	17	53
M5	13	16	12	41
M6	5	3	5	13
M7	3	1	3	7
Sex				
Male	75	65	72	212
Female	46	56	52	154
PS				
0	110	113	103	326
1	6	8	9	23
2	5	0	4	9
3			6	6
4			2	2

patients who had received additional chemotherapy during the first course of the individualized therapy, 22 (41.5 %) achieved CR (Table 2). There was no statistically significant difference in CR rates between the two groups regarding cytogenetics, gender, age, PS or FAB classification (data not shown).

The individualized group received significantly larger dosages of BHAC ($p < 0.001$) and DNR ($p < 0.001$) during the first course of induction therapy (Table 3). Myelosuppression judged by the period of leukocyte count <1,000/μL after the first course of induction therapy was significantly severer in the individualized group ($p = 0.040$) (Table 4). Early death within 30 days occurred in 5 (4.1 %) patients in the fixed-scheduled group and 4 (3.3 %) in the individualized group. There was no statistically significant difference in the incidence of complications between the two groups (Table 4).

Significant prognostic factors for the achievement of CR in all patients were cytogenetic risk group and gender (Table 2). Eighteen (90 %) of 20 patients with favorable risk cytogenetics, 120 (65.6 %) of 183 patients with intermediate risk, and 7 (25 %) of 28 with adverse risk achieved CR, respectively ($p < 0.0001$). Seventy-four (72.5 %) of 102 female patients achieved CR, while 76 (54.5 %) of 140 male patients attained it ($p = 0.0048$). These 2 factors were statistically significant and independent prognostic factors by the multivariate analysis (Table 5). Since this randomized study only included elderly patients who had met the eligibility criteria and agreed to enter the study, PS was 0 in 223 patients (92 %), 1 in 14 (6 %) and 2 in 5 (2 %). Paradoxically, patients with PS 1 or 2 had higher CR rate (84.2 %) compared with those with PS 0 (60.1 %) by the univariate analysis ($p = 0.0478$), but the difference was not statistically significant by the multivariate analysis ($p = 0.0998$).

Table 2 Response to induction therapy

Response by induction	Fixed-scheduled	Individualized	Total	*p* value
CR	73 (60.3 %)	77 (63.6 %)	150 (61.9 %)	0.6913
Non CR	48 (39.7 %)	44 (36.4 %)	92 (38.0 %)	
CR after first course	56 (46.3 %.)	56 (46.3 %)	114 (47.1 %)	
CR after second course	17 (14.0 %)	21 (17.4 %)	39 (16.1 %)	
Response by age group	65–69 years	70–74 years	75–79 years	
CR	64/106 (61.0 %)	56/90 (62.2 %)	30/47 (63.8 %)	0.9429
Response by PS	PS 0	PS 1	PS 2	
CR	134/223 (60.1 %)	11/14 (78.6 %)	5/5 (100.0 %)	0.0804
Response by PS	PS 0	PS 1 + 2		
CR	134/223 (60.1 %)	16/19 (84.2 %)		0.0478
Response by gender	Male	Female		
CR	76/140 (54.3 %)	74/102 (72.5 %)		0.0048
Response by cytogenetic risk[a]	Favorable	Intermediate	Adverse	
CR	18/20 (90.0 %)	120/183 (65.6 %)	7/28 (25.0 %)	<0.0001

[a] Cytogenetic data of 11 patients were not available

Table 3 Total administered dosage of behenoyl cytarabine (BHAC) and daunorubicin (DNR)

	BHAC (mg/m^2)		DNR (mg/m^2)	
	Average	Mean (range)	Average	Mean (range)
First course				
Fixed-scheduled (n = 121)	1,605	1,600 (200–3,000)	109	120 (40–240)
Individualized (n = 121)	1,851	1,600 (160–3,840)	139	120 (12–440)
p value	<0.001		<0.001	
Second course				
Fixed-scheduled (n = 42)	1,633	1,600 (1,600–2,400)	106	105 (60–180)
Individualized (n = 44)	1,732	1,600 (160–4,200)	123	120 (12–315)
p value	0.234		0.026	

Table 4 Toxicity during induction therapy

	Fixed-scheduled (n = 121)	Individualized (n = 121)	
Leukopenia			
G3/4	119 pts. (98.3 %)	117 pts. (96.7 %)	p = 0.513
G4	107 pts. (88.4 %)	111 pts. (91.7 %)	
Median duration of leucocytes <1,000/μl in G4 pts.			
1st course	14 days (2–52)	17 days (2–78)	p = 0.04
2nd course	15.5 days (2–32)	17.5 days (2–35)	p = 0.24
Use of G-CSF			
1st course	40 pts. (33.1 %)	44 pts. (36.4 %)	p = 0.686
2nd course	15 pts. (34.9 %)	11 pts. (24.4 %)	p = 0.352
Hemorrhage (CNS, pulmonary, GI): G3/4,	3 pts. (2.5 %)	3 pts. (2.5 %)	p = 1
Infection: G3/4	13 pts. (10.7 %)	11 pts. (9.1 %)	p = 0.72
Febrile neutropenia: G3/4	45 pts. (37.2 %)	48 pts. (39.7 %)	p = 0.696

Table 5 Multivariate analysis for achievement of complete remission and overall survival

Variable	Classification	No. of patients	Odds ratio	95 % CI	p value
Multivariate analysis for complete remission					
Treatment group	Fixed/individualized	115/116	0.973	0.543–1.744	0.9263
Age		231	1.000	0.925–1.081	0.9979
Sex	Male/female	133/98	2.192	1.200–4.003	0.0106
PS	0/1 + 2	213/18	3.065	0.808–11.634	0.0998
Cytogenetic risk	Favorable/Adverse	20/28	28.435	5.108–158.288	0.0001
	Intermediate/Adverse	183/28	4.764	1.878–12.086	0.0010
Multivariate analysis for overall survival					
Treatment group	Fixed/Individualized	115/116	1.037	0.751–1.430	0.8264
Age		231	1.003	0.961–1.047	0.8924
Sex	Male/female	133/98	0.747	0.530–1.052	0.0952
PS	0/1 + 2	213/18	0.833	0.365–1.902	0.6650
Cytogenetic risk	Favorable/adverse	20/28	0.390	0.185–0.820	0.0130
	Intermediate/adverse	183/28	0.422	0.261–0.680	0.0004

Analyzed in 231 patients by excluding 11 patients whose cytogenetic data were not available

Of 150 patients who had achieved CR, 63 patients were randomly assigned to receive ubenimex during 3 courses of consolidation therapy, plus 3 more months thereafter, and 60 received no ubenimex. All courses of consolidation therapy were administered to 65 (84.4 %) of 77 patients in the individualized group and 58 (79.5 %) of 73 patients in the fixed-scheduled group (p = 0.5248). There was no significant difference between patients receiving ubenimex or none, regarding myelosuppression and non-hematological toxicity, as well as complications during the consolidation therapy.

At a median follow-up of 39 months (range 2–76 months), predicted 4-year OS was 18.3 % for the fixed-scheduled group

and 17.1 % for the individualized group ($p = 0.807$) (Fig. 2a), and predicted 4-year RFS for patients who had achieved CR was 8.8 % for the former group and 17.9 % for the latter ($p = 0.467$) (Fig. 2b). Significant prognostic factors for OS were cytogenetic risk group and gender (Table 5). Predicted 2-year OS for patients with favorable cytogenetic risk was 56.4 %, while that for patients with intermediate risk was 35.8 % and for patients with adverse risk was 12.6 % ($p < 0.0001$ both for favorable and intermediate risk groups vs. adverse risk group) (Fig. 3). Predicted 4-year OS for female patients was 24.4 %, while that for male patients was 13.5 % ($p = 0.0018$) (Fig. 4). By the multivariate analysis, cytogenetic risk group was a significant prognostic factor ($p < 0.0001$), but the significance regarding gender was marginal ($p = 0.0106$) (Table 5). It is of note that there was no significant difference in OS among patients of age 65–69, 70–74 and 75–79 (Fig. 5). For 124 patients in the non-randomized observation cohort, predicted 1-, 2-, 3- and 4-year OS was 46.5, 33.7, 26.5 and 21.6 %, respectively, which did not differ from the randomized cohorts (Fig. 2a).

Among patients who had obtained CR, predicted 4-year OS was 32.3 % for 63 patients in the ubenimex group, and 18.7 % for 60 patients in the control group ($p = 0.111$) (Fig. 6a). Predicted 4-year RFS was 16.4 % for the former group and 10.4 % for the latter, in favor of the ubenimex group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test) (Fig. 6b).

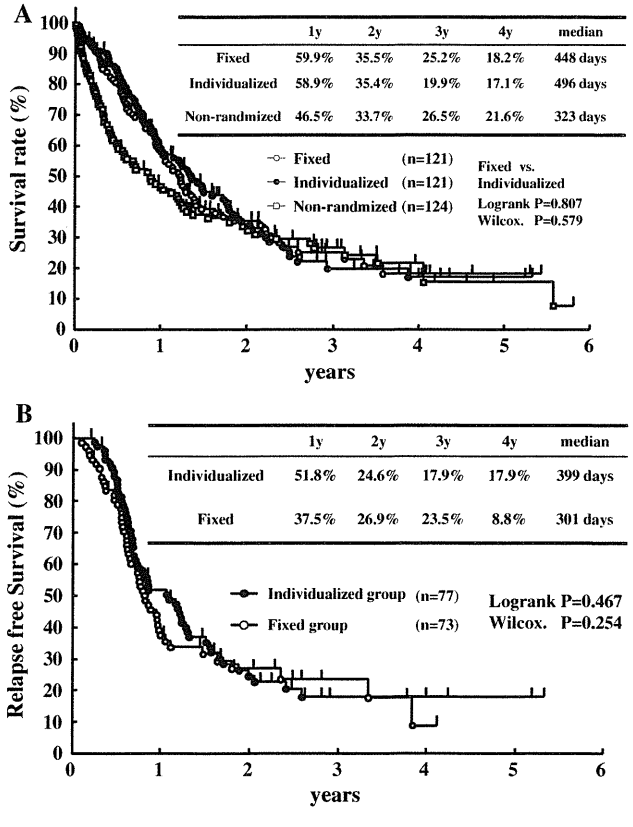

Fig. 2 Overall survival and relapse-free survival. Overall survival rate in three groups (**a**). There was no significant difference in each group. Relapse-free survival in fixed and individualized group (**b**). There was no significant difference in each group

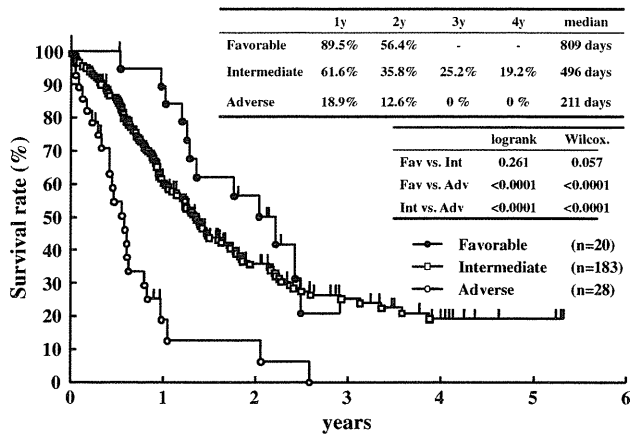

Fig. 3 Overall survival according to cytogenetics. Survival rate decreases down according to cytogenetics group

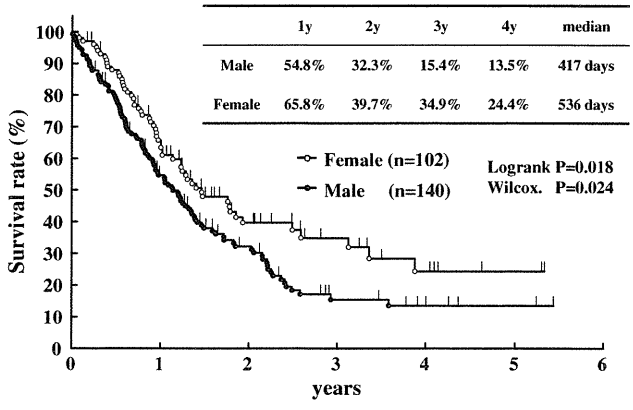

Fig. 4 Overall survival according to gender. There was significant difference between male and female

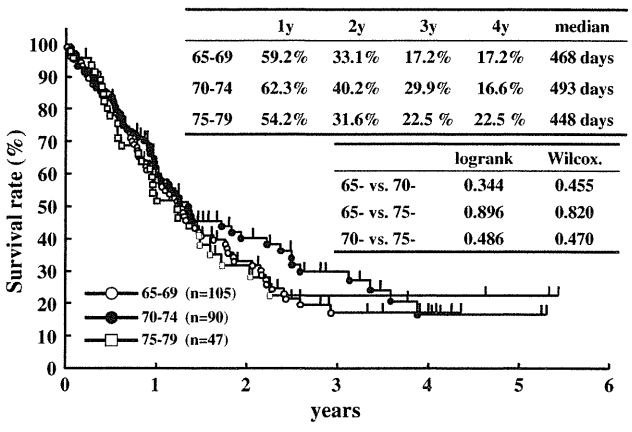

Fig. 5 Overall survival according to age. There was no significant difference in three age groups

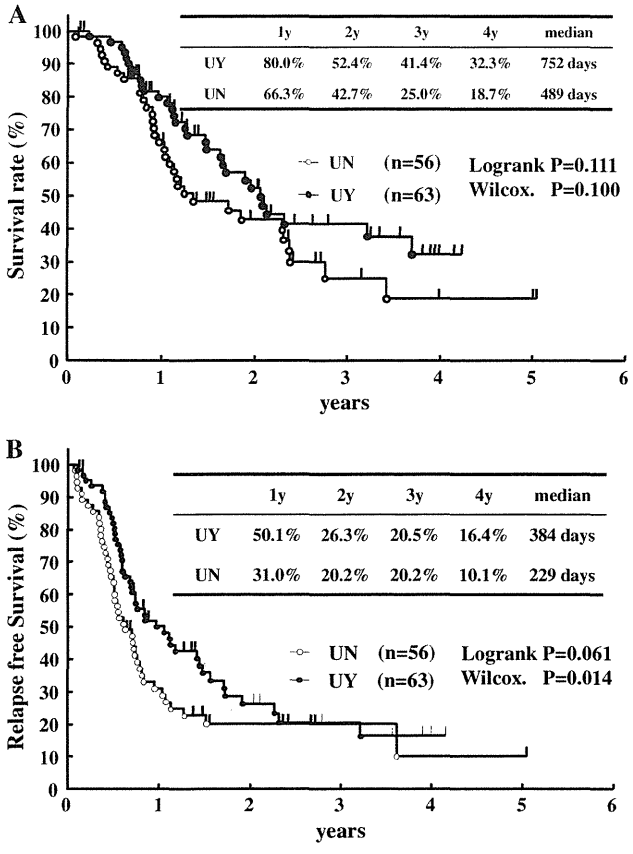

Fig. 6 Overall survival and relapse free survival for with ubenimex group and without ubenimex group. Overall survival (**a**). There was no significant difference between two groups. Relapse-free survival (**b**). There was significant difference by Wilcoxon analysis. UY is with ubenimex group, UN is without ubenimex group

Discussion

Aging generally causes comorbidity, poor performance status, decreased immune competency, deficient stem cell reservoir in bone marrow and so on, and inevitably puts patients at a great disadvantage for receiving intensive chemotherapy. Additionally, elderly AML is biologically associated with higher frequency of adverse karyotypes such as complex abnormalities and aberrations of chromosomes 5 or 7, MDR1 expression, antecedent MDS and secondary AML. Thus, the treatment outcome of elderly patients with AML is much poorer than that of younger patients, when treated with currently available intensive therapy using cytotoxic drugs [1–6].

Recently, HOVON/AMLSG/SAKK group reported that escalation of the dose of DNR to twice the conventional dose to elderly patients of age 60–83 (median 67) with AML or high-risk refractory anemia resulted in higher CR rate (64 vs. 54 %, $p = 0.0002$) without additional toxic effects, but that OS did not differ significantly between the two groups. Subset analysis, however, revealed that only patients of age 60–65 in the escalated-treatment group had significantly higher CR rate (73 vs. 51 %), event-free survival (29 vs. 14 %), and OS (38 vs. 23 %) than patients of the same age range in the conventional dose group, indicating that there was no advantage in the escalated treatment to patients older than 65 [21].

In Japan, where people enjoy the longest life expectancy in the world, JALSG has regarded patients as elderly when they were 65 years or older, since the AML95 study started in 1995, after the analysis of the treatment outcomes of preceding AML87, AML89 and AML92 studies in which patients of age 65 or older were included [9–11]. Thus, even in the HOVON/AMLSG/SAKK study, the question of recommendable treatment for elderly patients older than 65 remains unsettled.

Most drug therapies are generally carried out in a response-oriented and individualized manner, and physicians adjust dosage and treatment period depending on the response of patient's symptoms to administered drugs. However, cancer chemotherapy is generally carried out by fixed dosage and period, because the nadir of myelosuppression, the most important toxic effect of cytotoxic drugs, appears 7–10 days after the discontinuation of drugs. Myelosuppression is usually judged by leukocyte or platelet counts in the peripheral blood, but, if it is judged by bone marrow itself, it is possible to obtain information on myelosuppression directly and earlier.

We attributed the higher CR rates of our previous JALSG studies for adult AML: AML87 [9], AML89 [10] and AML92 [11], to response-oriented individualized therapy, which administered highly intensive but not too toxic doses of anti-leukemia drugs, especially DNR. Disappointingly, however, a prospective randomized study for AML of younger patients of age <65, the JALSG-AML95 failed to demonstrate that response-oriented individualized therapy was superior to the fixed-scheduled therapy, although IDR instead of DNR was used in combination with Ara-C in this study [15]. Both regimens resulted in very high CR rates: 79 and 82 %, respectively, but leukocytopenia was significantly severer and its duration significantly longer, and early death within 30 days tended to occur more frequently in the individualized group. We speculated that, if DNR instead of IDR had been used, the CR rate of the fixed-scheduled group might have been lower like around 70 % as reported from other large scale multicenter studies.

In the present study with elderly patients, we again prospectively compared a fixed-scheduled therapy with a response-oriented individualized therapy, utilizing DNR and BHAC. BHAC has been chosen because this analogue of Ara-C is administered by 3-h infusion, instead of 24-h continuous infusion required for Ara-C, and thus is more conveniently given especially to elderly patients, and also

because BHAC in combination with DNR, 6MP and PSL produced over 70 % CR rates in adult AML in the previous JALSG studies.

Again, however, we could not demonstrate that the response-oriented individualized therapy was not inferior to the fixed-scheduled therapy. CR rate and OS were almost the same in both groups. Patients in the individualized therapy group, being given additional drugs on day 8 and thereafter, showed severer myelosuppression, but the 30-day mortality rates were almost the same in both groups.

Ubenimex is a small molecule inhibitor of leucine aminopeptidase and has various immunomodulatory properties via macrophage or T cell activation. A myeloid lineage marker, CD 13, has been identified as aminopeptidase N36. Ubenimex inhibits aminopeptidase N, and increases the sensitivity of leukemia cells to apoptosis through the inhibition of cell-surface aminopeptidase N activities by hampering the degradation of endothelial cell-derived interleukin 8 [16, 22–24]. In the JALSG AML89 study for younger patients with AML, however, we could not demonstrate that ubenimex given after the end of maintenance chemotherapy improved DFS of AML patients [25]. In this study for elderly AML, ubenimex given orally during and after the consolidation therapy did not clearly improve OS, although RFS in the ubenimex group was longer than that in no-ubenimex control group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test).

Cytogenetic risk factor was the most important prognostic factor in this study. Although the number of cytogenetically favorable risk group was small (9 %), 90 % achieved CR and predicted 2-year OS was 56 %. Of patients with intermediate risk cytogenetics, 66 % achieved CR and predicted 4-year OS was 19 %. Of patients with adverse risk cytogenetics, only 25 % achieved CR and predicted 3-year OS was 0 %. Thus, elderly patients with favorable and intermediate risk karyotypes seemed to be benefitted from the present chemotherapy, but not those with adverse risk cytogenetics.

One interesting observation from this study was that female elderly patients had significantly higher CR rate and better OS compared with male patients. Although our female patients tended to have less adverse risk cytogenetics, gender was an independent significant factor for the achievement of CR ($p = 0.0106$) and marginal one for OS ($p = 0.0952$) by the multivariate analysis. In our past adult AML studies, there has been no such observation. ECOG reported that female gender was one of the independent prognostic factors to predict a long-term survival of more than 3 years among 1,414 adult AML patients, but karyotypes were not included in their analysis [26]. German Study Alliance Leukemia recently proposed a novel prognostic model for elderly patients with AML, based on the data of 909 patients entered into the prospective trial, but female gender was not a prognostic factor in achievement of CR, or in OS, either [27]. On the other hand, in childhood leukemia, female patients generally have better prognosis, although no clear explanation has been provided so far. The average remaining life expectancy of Japanese female of age 65 in 2002 was 22.4 years which is 5 years longer than Japanese male of the same age (17.4 years), and this may apply to leukemia patients. However, the higher CR rate is not explainable by this statistics of average life expectancy. Another notable observation was that age was not prognostic factor in the present setting. If patients are eligible for rather strict inclusion criteria as in this study, chronological age alone should not be regarded as a single bad prognostic factor.

In conclusion, we could not demonstrate that the response-oriented individualized therapy gave a better treatment outcome in elderly AML of age 65 or older. Ubenimex given concomitantly during consolidation therapy and thereafter showed a marginal benefit in RFS, but was not impressive. The treatment of elderly AML is still being explored, and new effective therapeutic drugs, especially pathogenic molecule-specific target drugs, are desperately awaited for the treatment of this leukemia, which is increasing in number all over the world.

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

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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.

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

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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.

Table 1. Patient, Disease, and Transplantation Characteristics According to Stem Cell Source and Number of Mismatched Loci

	Bone Marrow Transplant			
	Class II One Locus Mismatch	Class I One Locus Mismatch	Two Loci Mismatch	Cord Blood Transplantation
	N (%)	N (%)	N (%)	N (%)
Number of transplantations	248	424	356	351
Patient age at transplantation				
Median (range)	36 (16-60)	34 (16-67)	34 (16-59)	37 (16-58)
Patient sex				
Male	151 (61)	241 (57)	210 (59)	162 (46)
Female	97 (39)	183 (43)	146 (41)	189 (54)
Sex matching				
Matched	145 (58)	268 (63)	217 (61)	170 (48)
Male to female	52 (21)	82 (19)	73 (21)	97 (28)
Female to male	50 (20)	71 (17)	64 (18)	84 (24)
Unknown	1 (<1)	3 (1)	2 (1)	0 (0)
Diagnosis				
AML	135 (54)	204 (48)	172 (48)	193 (55)
ALL	78 (31)	149 (35)	135 (38)	113 (32)
MDS	35 (14)	71 (17)	49 (14)	45 (13)
Disease status				
Standard	124 (50)	214 (50)	168 (47)	147 (42)
Advanced	114 (46)	195 (46)	169 (47)	174 (50)
Unknown	10 (4)	15 (4)	19 (5)	30 (9)
ABO matching				
Matched	119 (48)	184 (43)	153 (43)	114 (32)
Minor mismatch	53 (21)	108 (25)	85 (24)	99 (28)
Major mismatch	67 (27)	116 (27)	97 (27)	73 (21)
Bidirectional	8 (3)	12 (3)	14 (4)	64 (18)
Unknown	1 (<1)	4 (1)	7 (2)	1 (<1)
HLA-mismatched number and direction				
Matched				20 (6)
One locus mismatched				87 (25)
HVG direction	16 (6)	38 (9)		8 (9)
GVH direction	17 (7)	30 (7)		8 (9)
Both directions	215 (87)	356 (84)		71 (82)
Two loci mismatched				244 (70)
Two HVG direction			4 (1)	2 (1)
One HVG direction and one GVH direction			6 (2)	4 (2)
Two GVH direction			4 (1)	3 (1)
One both directions and one HVG direction			42 (12)	40 (16)
One both directions and one GVH direction			29 (8)	28 (11)
Two both directions			271 (76)	167 (68)
No. of nucleated cells infused ($\times 10^7$/kg)				
Median	25.0	24.5	23	2.46
Range	2.40-59.8	2.10-97.5	1.5-66.0	1.41-6.01
Preparative regimen				
CY + TBI	94 (38)	168 (40)	151 (42)	109 (31)
CY + CA + TBI	46 (19)	78 (18)	74 (21)	124 (35)
CY + BU + TBI	20 (8)	39 (9)	27 (8)	15 (4)
Other TBI regimen	45 (18)	70 (17)	61 (17)	80 (23)
BU + CY	34 (14)	54 (13)	30 (8)	21 (6)
Other non-TBI regimen	9 (4)	15 (4)	13 (4)	2 (1)
GVHD prophylaxisis				
Cycrosporine A + sMTX	87 (35)	221 (52)	150 (42)	213 (61)
Cyclosporine A ± other	1 (<1)	5 (1)	5 (1)	24 (7)
Tacrolimus + sMTX	152 (61)	191 (45)	193 (54)	76 (22)
Tacrolimus ± other	8 (3)	5 (1)	6 (2)	35 (10)
Others	0 (0)	2 (<1)	2 (<1)	3 (1)

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; BU, oral busulfan; CA, citarabine; CY, cyclophosphamide; GVH, graft-versus-host; HVG, host-versus-graft; MDS, myelodysplastic syndomes; sMTX, short-term methotrexate.

Outcome

OS and RFS

OS and RFS for CB recipients were similar when compared with that of single-HLA-DRB1-mismatched BM recipients (relative risk [RR] = 0.85, 95% confidence interval [CI], 0.68-1.06; P = .149 for OS and RR = 0.97, 95% CI, 0.92-1.35; P = .747) (Table 2).

The adjusted probabilities of survival at 3 years posttransplantation of CB recipients (47%) were not different from those of single HLA-DRB1 mismatched BM recipients (41%; P = .19) or single HLA class I-mismatched BM recipients (47%; P = .96), but superior to those of 6 of 8 BM recipients (38%; P = .014) (Figure 1A). Figure 1B shows adjusted RFS curves (42% for CB recipients, 36% for single HLA-DRB1-mismatched BM, 44% for single HLA class I-mismatched BM, and 36% for 6 of 8 BM recipients, at 3 years posttransplant) (P values of comparison between CB and single HLA-DRB1-mismatched BM, CB, and single HLA

Table 2. Multivariate Analyses of Overall Survival, Relapse-Free Survival, Relapse, and Transplant-Related Mortality

	Degree of HLA Mismatch	N	Overall Survival			Relapse-Free Survival			Relapse			Transplant-Related Mortality		
			RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value	RR	(95% CI)	P value
Bone marrow transplant	Single DRB1 (7/8)	248	1.00			1.00			1.00			1.00		
	Single A or B (7/8)	137	0.84	(0.64-1.11)	.216	0.82	(0.63-1.08)	.158	0.65	(0.41-1.01)	.056	1.07	(0.77-1.49)	.698
	Single C (7/8)	287	0.89	(0.72-1.12)	.324	0.86	(0.69-1.07)	.170	0.60	(0.41-0.87)	.007	1.13	(0.86-1.48)	.391
	C + DRB1 (6/8)	144	0.97	(0.74-1.27)	.831	0.95	(0.73-1.24)	.726	0.76	(0.49-1.17)	.208	1.10	(0.78-1.55)	.600
	A/B + C (6/8)	122	1.22	(0.94-1.59)	.143	1.15	(0.88-1.49)	.300	0.70	(0.44-1.10)	.12	1.42	(1.03-1.96)	.032
	Other two loci (6/8)	90	1.25	(0.92-1.68)	.146	1.13	(0.84-1.53)	.409	0.60	(0.35-1.02)	.061	1.48	(1.03-2.13)	.035
Cord blood transplant		351	0.85	(0.68-1.06)	.149	0.97	(0.92-1.35)	.747	1.28	(0.93-1.76)	.125	0.68	(0.50-0.92)	.011

RR indicates relative risk; CI, confidence interval.
Adjusted by patient age at transplantation >40 versus ≤40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplantation, cyclophosphamide and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and CB and 6 of 8 BM recipients were 0.80, 0.12, and 0.43, respectively).

Relapse and TRM

There was no significant increase of relapse rates among CB recipients when compared with DRB1 single-mismatched BM recipients (RR = 1.28, 95% CI, 0.93-1.76; $P = .125$). The risk of TRM was lower in CB recipients compared with that of single HLA-DRB1-mismatched BM recipients (RR = 0.68, 95% CI, 0.50-0.92; $P = .011$) (Table 2). The risk of TRM was also lower in CB recipients when compared with 6 of 8 BM recipients (RR = 0.52, 95% CI, 0.39-0.68; $P < .001$).

Hematologic recovery

Neutrophil and platelet recovery was inferior in CB recipients, as shown in Table 3 (RR = 0.50, 95% CI, 0.42-0.60; $P < .001$ for neutrophil recovery, RR = 0.52, 95% CI, 0.42-0.63; $P < .001$ for platelet recovery).

Acute GVHD and chronic GVHD

The risk of grade 2 to 4 or severe (grades 3-4) aGVHD was lower in CB recipients than that of single HLA-DRB1-mismatched BM recipients (RR = 0.55, 95% CI, 0.42-0.72; $P < .001$ for grade 2 to 4 aGVHD and RR = 0.43, 95% CI, 0.27-0.58; $P < .001$ for severe aGVHD) (Table 4). Unadjusted cumulative incidence of severe aGVHD was 9% for CB, 19% for single HLA-DRB1-mismatched BM, 18% for single HLA

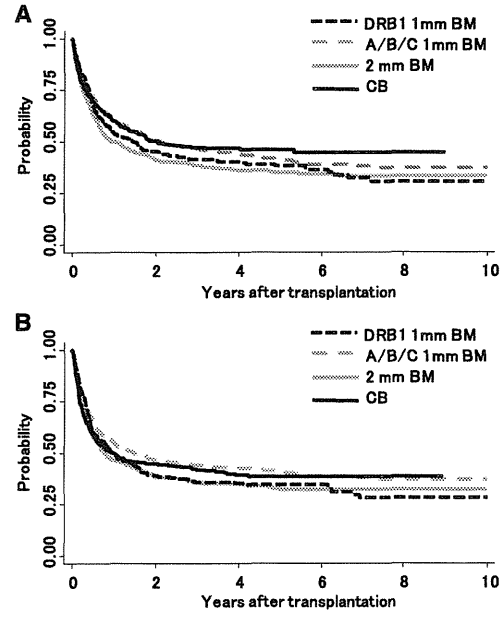

Figure 1. Adjusted probabilities of OS (A) and RFS (B). The adjusted 3-year probabilities of OS for unrelated cord blood recipients, single-HLA-DRB1-mismatched unrelated bone marrow (UBM) recipients, single-HLA-class-I-mismatched UBM, and 6 of 8 UBM recipients were 47%, 41%, 47%, and 38%, respectively (A). The adjusted 3-year probabilities of RFS were 42%, 36%, 44%, and 36%, respectively (B).

Table 3. Multivariate Analyses of Neutrophil and Platelet Recovery

			Neutrophil Recovery			Platelet Recovery		
	Degree of HLA Mismatch	N	RR	(95% CI)	*P* value	RR	(95% CI)	*P* value
Bone marrow transplantation	Single DRB1 (7/8)	248	1.00			1.00		
	Single A or B (7/8)	137	1.31	(1.04-1.65)	.021	1.31	(1.01-1.70)	.039
	Single C (7/8)	287	1.19	(0.98-1.43)	.069	0.98	(0.79-1.21)	.840
	C + DRB1 (6/8)	144	0.96	(0.77-1.20)	.735	0.79	(0.62-1.02)	.065
	A/B + C (6/8)	122	1.14	(0.89-1.45)	.307	0.84	(0.63-1.13)	.255
	Other two loci (6/8)	90	0.89	(0.68-1.14)	.346	0.80	(0.58-1.10)	.174
Cord blood transplantation		351	0.50	(0.42-0.60)	<.001	0.52	(0.42-0.63)	<.001

RR indicates relative risk; CI, confidence interval.

Adjusted by patient age at transplantation >40 versus <40, patient sex, donor-patient sex mismatch versus matched, ABO major mismatch versus others, advanced versus standard disease status at transplant, cyclophosphamide, and total-body irradiation or busulfan and cyclophosphamide for conditioning versus other conditioning regimen, and cyclosporine-based versus tacrolimus-based prophylaxis against graft-versus-host disease.

class I-mismatched BM, and 22% for 6 of 8 BM at 100 days posttransplantation ($P < .001$ between CB and single HLA-DRB1-mismatched BM) (Figure 2A).

Among recipients who survived at least 100 days posttransplantaton, the risk of developing cGVHD and extensive-type cGVHD was not significantly increased in all HLA disparity groups of CB recipients when compared with that of HLA-DRB1-allele/antigen-mismatched BM recipients (RR = 1.36, 95% CI, 0.99-1.88; $P = .057$ for cGVHD, and RR = 0.86, 95% CI, 0.55-1.34; $P = .500$ for extensive-type cGVHD). The unadjusted cumulative incidence of extensive-type cGVHD was 17% for CB recipients, 20% for single HLA-DRB1-mismatched BM, 25% for single HLA class I-mismatched BM, and 30% for 6 of 8 BM recipients at year posttransplantation ($P = .34$ between CB and single HLA-DRB1-mismatched BM) (Figure 2B).

DISCUSSION

Our main objective was to compare OS after transplantation of UCBT and single-HLA-mismatched UBMT and to provide useful data for selection of an appropriate donor and graft source in second stem cell source/donor selection for adults with hematologic malignancy. To the best of our knowledge, this is the first study to involve mismatched allele/antigen-specific analyses including CB for the process of donor selection. Our results suggest that 0 to 2 HLA-mismatched UCB is a reasonable second alternative of choice for adult patients with leukemia, with similar survival to that of single DRB1-mismatched or other 7 of 8 UBM recipients, the current first choice for second alternative donor/stem cells.

Neutrophil and platelet recovery was slower in CB recipients than BM recipients, consistent with the results of previous reports [7-10,12]. This is the major limitation of the use of UCB, and several strategies have been studied to reduce the neutropenic period, such as screening for patients' pretransplantation anti-HLA antibodies and their specificity, transplantation of 2 UCB units if a single UCB unit with an adequate cell dose is not available, or direct infusion of UCB into bone marrow [22-26].

Despite higher HLA disparity at the antigen level (69% 2 antigen mismatch, 25% antigen mismatch, and 6% matched), UCB recipients showed lower incidence of severe aGVHD than single DRB1-mismatched UBM recipients, consistent with other reports that compared UCB with single-mismatched UBM (7 of 8) [8,11,12]. In our study, tacrolimus and short-term methotrexate were used preferentially in BM recipients, whereas cyclosporine A was used in 68% of CB recipients. Prior studies have shown reduced severe aGVHD with tacrolimus, and this difference may have underscored the improved aGVHD control of UCB over mismatched BM in unadjusted analyses [27,28]. It is likely that decreased risk of grade 2 to 4 aGVHD in UCB recipients contributed to decreased risk of TRM among UCB recipients.

Increasing the number of HLA mismatches from 7 of 8 to 6 of 8 was associated with an approximately 10% reduction in survival in UBM recipients, which was quite similar to the results from the National Marrow Donor Program [3]. Because we eliminated data from the first 3 pioneering years of unrelated BMT, most of the bone marrow recipients and donors were allele-typed for at least HLA-A, -B, and -DRB1 before transplantation. Survival outcomes of single class I mismatch were not significantly different from those of single class II mismatch in the current analyses. We believe that allele typing of HLA-A, -B, and -DRB1 before transplantation led to better selection of the donor compared with that in the first several years of UBMT. This study includes a large number of fully typed BM and CB recipients, but there are limitations. The choice of stem cell source is influenced by many unmeasured factors that can affect outcome. It is also influenced by the availability of acceptable HLA disparity for unrelated donors and mainly cell dose for cord blood units. Although we have adjusted for known risk factors and disparities between groups, we cannot rule out the influence of potential selection bias, which can only be excluded in a randomized controlled trial. Transplantation years