myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), or malignant lymphoma (ML) between January 1997 and August 2005 at the Institute of Medical Science, University of Tokyo. T-cell depletion was not performed in either group. Patients qualified as being standard risk if they were in first or second complete remission (CR), had chronic-phase CML or refractory anemia MDS, or had no high-risk cytogenetics (eg, ALL with t(4;11) or t(9;22), or AML with complex karyotype, −5, del(5q), −7, or abnormalities of 3q). Patients in third CR, in relapse, with CML beyond chronic phase, or with high-risk cytogenetics were classified as being high risk. Patients receiving BMT, PBSCT, or CBT as a second transplant following relapse after a first allogeneic transplantation were excluded. Median follow-up was 32 months (range, 1-110 months; 39 survivors and 32 censored) for BMT and 22 months (range, 0-91 months; 72 survivors and 28 censored) for CBT recipients ($P = .77$). The clinical protocol was approved by the institutional review board of the Institute of Medical Science, University of Tokyo, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

HLA typing and donor selection

HLA-A and HLA-B antigens were identified by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using polymerase chain reaction sequence-specific primers (PCR-SSPs). Patients without a suitable closely HLA-matched related donor, namely, with 5 of 6 or 6 of 6 matching HLA loci, were eligible for cord blood transplantation as a first treatment option, because most patients eligible for allogeneic stem-cell transplantation were thought to have insufficient time for an unrelated bone marrow donor search and early timing of transplantation was preferable. On the other hand, if those patients had any type of anti-HLA antibody, we generally attempted to locate bone marrow grafts from unrelated donors because most cord blood grafts were HLA mismatched and the predictable risk of poor engraftment results after cord blood transplantation. All cord blood grafts were evaluated by HLA-A and HLA-B typing serologically, by HLA-DRB1 typing at high resolution, and by nucleated cell counts. Preferred cord blood units matched 4 of 6 to 6 of 6 HLA loci and contained a minimal cell count of 1.5×10^7 nucleated cells/kg body weight before freezing. T-lymphocyte depletion was not performed on cord blood or bone marrow grafts.

Conditioning regimen, GVHD prophylaxis, and supportive care

All patients received a total body irradiation (TBI)–containing myeloablative pretransplantation conditioning regimen of 12 Gy, fractionated in 4 or 6 doses. The TBI + cytosine arabinoside (Ara-C: total dose 24 g/m^2) combined with G-CSF (lenograstim) regimen[13-15] was chosen for patients with myeloid leukemias who had an HLA-matched related donor (n = 31). Additionally, cyclophosphamide (CY) was administered to patients who received transplants from HLA-mismatched cord blood donors or HLA-mismatched related donors while reducing the Ara-C dose to a total dose of 12 g/m^2 (n = 82) as reported previously.[6] CY was avoided in favor of 120 mg/m^2 fludarabine (n = 6) in the case of recipients who had risk of organ dysfunction, especially in the heart. Thirty-one patients received TBI + CY (n = 23) or TBI + CY + one cytotoxic drug (12 g/m^2 Ara-C in 6 patients, 60 mg/kg etoposide in 1 patient, or 300 mg/m^2 thiotepa in 1 patient). Fifteen patients received TBI + 60 mg/kg etoposide. Six additional patients received TBI + 90 mg/m^2 fludarabine + one drug (12 g/m^2 Ara-C in 3 patients or 140 mg/m^2 melphalan in 3 patients) (Table 1).

One hundred and sixty-three (95%) of all 171 patients received a standard cyclosporine (CsA) and methotrexate (MTX) combination as GVHD prophylaxis. CsA was administered daily from day −1 at 3 mg/kg per day intravenously and MTX at 15 mg/m^2 intravenously on day 1, followed by 10 mg/m^2 on days 3 and 6. MTX on day 11 was given only to patients receiving HLA-mismatched bone marrow or peripheral blood from related donors. Six patients received only CsA, 1 received tacrolimus (FK-506) combined with a short course of MTX, and 1 received CsA plus mycophenolate mofetil (MMF). Once oral intake could be tolerated, patients were administered oral CsA at a dose ratio of 1:2.5, in 2 divided doses/d based on the last intravenous dose. In the absence of GVHD, CsA was tapered beginning between weeks 6 and 9 until it could be discontinued, depending on the degree of GVDH severity. Corticosteroid-based treatment was considered when grade II or higher severe acute GVHD occurred (1 to 2 mg/kg).

The supportive-care regimen, including prophylaxis, for infection was the same as previously reported.[6] All patients after cord blood transplantation and 60 of 71 after bone marrow transplantation/peripheral blood stem-cell transplantation received G-CSF (lenograstim, 5 μg/kg per day, intravenous infusion) starting on day 1 until durable granulocyte recovery was achieved. The same supportive care, except for the G-CSF administration, was given to both groups.

End points, definitions, and assessments of hematopoietic recovery, GVHD, TRM, disease relapse, and DFS

We focused on hematologic recovery, acute and chronic GVHD, TRM, disease relapse, and DFS after unrelated cord blood transplantation compared with related bone marrow transplantation/peripheral blood stem-cell transplantation. The primary measure of hematopoietic recovery was the time required for myeloid and platelet recovery. The myeloid-cell recovery time was defined as the first of 3 consecutive days during which the absolute neutrophil count in the blood was at least 0.5×10^9/L.[3] Platelet recovery time was achieved on the first of 3 days when the platelet count was higher than 2×10^9/L (or 5×10^9/L) without transfusion support. Primary engraftment failure was defined as the absence of donor-derived myeloid cells on the day of death, the day of relapse, or day 60 in patients surviving beyond day 28 after transplantation. Patients were also defined as having had primary engraftment failure when either a second allogeneic transplantation before donor-derived myeloid recovery or reconstitution with autologous cells was required. Chimerism was evaluated by fluorescence in situ hybridization for the Y chromosome or quantitative PCR analysis for microsatellite DNA markers. Acute GVHD was graded 0 to IV according to the criteria of Glucksberg et al,[16] and chronic GVHD was defined as none, limited, or extensive.[17] The incidence of and time to acute GVHD development were evaluated in patients surviving 21 days or longer with evidence of engraftment. Time to occurrence of any chronic GVHD disease was evaluated in patients surviving 100 days or longer after transplantation with allogeneic engraftment. TRM was defined as death from any cause except relapse. Relapse was defined by morphologic evidence of disease in peripheral blood, marrow, or extramedullary sites, or the recurrence and sustained presence of pretransplantation chromosomal abnormalities on cytogenetic analysis of bone marrow cells. Patients showing minimal residual disease (eg, the presence of bcr/abl RNA transcripts by PCR) were not classified as having relapsed. DFS was defined as survival in continuous CR.

Statistical analysis

The probability of DFS was estimated from the time of transplantation according to the Kaplan-Meier product limit method. Cumulative incidences were estimated for hematopoietic recovery, GVHD, TRM, and relapse in order to take competing risks into account. Associations between graft type and outcome were evaluated using Cox proportional hazard regression models. In addition to the hematopoietic stem-cell source, the following variables were considered as covariates: recipient age at transplantation; weight; status regarding cytomegalovirus (CMV, determined by serologic testing); recipient and donor sex; degree of ABO matching; degree of HLA matching; type (ALL, AML, CML, MDS, or malignant lymphoma) and pretransplantation duration of the underlying disease; disease status at transplantation (standard or high risk); conditioning regimen; GVHD prophylaxis used; use or nonuse of G-CSF during the first 7 days after transplantation; and time of transplantation (between 1997 and 2000, or 2001 and 2005). We used backward and stepwise procedures at a significance level of 5% to construct prognostic models, in which we tried to maintain the graft source (cord blood from an unrelated donor or bone marrow/peripheral blood from a related donor) as a variable until the final step of the procedures. The proportional hazard assumption of the Cox model was assessed essentially by a graphic approach. When groups were compared according to continuous covariates, we calculated the mean or

Table 1. Characteristics of patients and grafts

Characteristic	BMT or PBSCT recipient	CBT recipient	P*
Recipients, n	71	100	
Age, y			.83
Median	40	38	
Range	16-58	16-55	
Weight, kg			.01
Median	59	55	
Range	35-85	36-76	
Sex of donor and recipient, no. (%)			.69
Male/male	26 (37)	29 (29)	
Female/female	15 (21)	20 (20)	
Female/male	16 (23)	27 (27)	
Male/female	14 (20)	24 (24)	
CMV serologic status, no. (%)			.37
Negative	6 (9)	13 (13)	
Positive	64 (91)	87 (87)	
Diagnosis, no. (%)			.10
AML			
CR1, CR2	8 (11)	26 (26)	
Advanced	23 (32)	31 (31)	
ALL			
CR1, CR2	7 (10)	9 (9)	
Advanced	10 (14)	11 (11)	
CML			
CP	7 (10)	1 (1)†	
Advanced	4 (6)	5 (5)	
MDS			
RA	4 (6)	3 (3)	
Advanced	1 (1)	8 (8)	
ML			
CR1, CR2	1 (1)	5 (5)†	
Advanced	6 (9)	2 (2)	
Duration from diagnosis to transplantation, mo			.30
Median	15	17.5	
Range	2-177	2-223	
Conditioning, no. (%)			<.01
TBI + Ara-C/G-CSF	31 (44)	0	
TBI + Ara-C/G-CSF + CY	14 (20)	68 (68)	
TBI + CY	10 (14)	13 (13)	
TBI + other combination‡	16 (23)	19 (19)	
GVHD prophylaxis, no. (%)			.39
CsA	2 (3)	4 (4)	
CsA + sMTX	67 (94)	96 (96)	
FK506 + sMTX	1 (1)	0	
CsA + MMF	1 (1)	0	
G-CSF administration during first 7 d, no. (%)			<.01
Yes	60 (85)	100 (100)	
No	11 (15)	0	
Year of transplantation, no. (%)			<.01
1997 to 2000	50 (70)	21 (21)	
2001 to 2005	21 (30)	79 (79)	
No. of leukocytes for transplantation, $\times 10^7$/kg			<.01
Median	33.0	2.43	
Range	6.6-50	1.1-5.29	
No. of HLA-A, B, and DRB1 mismatches, no. (%)			<.01
0	54 (76)	0 (0)	
1	11 (15)	16 (16)§	
2	6 (8)	54 (54)§	
3	0	28 (28)§	
4	0	2 (2)§	
Extent of ABO match, no. (%)			<.01
Match	39 (55)	29 (29)	
Minor mismatch	15 (21)	31 (31)	

Table 1. Characteristics of patients and grafts (continued)

Characteristic	BMT or PBSCT recipient	CBT recipient	P*
Major mismatch	17 (24)	40 (40)	

Italicized *P* values are ___.

CMV indicates cytomegalovirus; AML, acute myelogenous leukemia; CR1 and CR2, 1st and 2nd complete remission, respectively: advanced, patients in third complete remission, in relapse, in CML beyond chronic phase, or who had high-risk cytogenetics were classified as high risk; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; CP, chronic phase; MDS, myelodysplastic syndrome; RA, refractory anemia; ML, malignant lymphoma; TBI, total body irradiation: Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; CsA, cyclosporine; sMTX, short-term methotrexate; FK506, tacrolimus; —, not applicable; and MMF, mycophenolate mofetil.

*The Chi-square test was used for categoric variables; the Mann-Whitney test was used for continuous variables. †One patient with ALL in chronic phase and non-Hodgkin lymphoma in first complete remission received cord blood for the treatment of both diseases and was categorized as standard risk.

‡All conditioning regimens included 12 Gy TBI. In BMT/PBSCT recipient group, 15 patients received TBI + etoposide (60 mg/kg). One patient received TBI + G-CSF–combined Ara-C + fludarabine (90 mg/m^2). In CBT recipient group, 5 patients received TBI + G-CSF–combined Ara-C + fludarabine (90 mg/m^2), 3 patients received TBI + Ara-C + fludarabine (90 mg/m^2), 6 patients received TBI + Ara-C + CY, 2 patients received TBI + one drug (etoposide or thiotepa) + CY, and 3 patients received TBI + fludarabine (90 mg/m^2) + melphalan (140 mg/m^2).

§In 16 patients receiving 1-HLA-antigen-mismatched cord blood grafts, 7 had a mismatch antigen in class I and 9 had a mismatch antigen in class II. In 54 patients receiving 2-antigen-mismatched grafts, 14 had mismatched antigens in class I, 37 had mismatched antigens both in classes I and II, and 3 had mismatched antigens in class II. In 28 patients receiving 3-antigen-mismatched grafts, 18 had 2 class I mismatched antigens and a class II mismatched antigen, and 10 had a class I mismatched antigen and 2 class II mismatched antigens.

median of each group, and the Student *t* test or Mann-Whitney *U* test was used. A Chi-square test was used to compare categoric covariates: SAS version 8.2 (SAS Institute, Cary, NC) and S Plus 2000 (Mathsoft, Seattle, WA) were used for all analyses. End points were calculated at the last contact, the date of the latest follow-up being March 1, 2006.

Results

Characteristics of patients and donors

The patients' age, sex, CMV serology, diagnosis, the ratio of standard-risk versus high-risk, duration from diagnosis to transplantation, and GVHD prophylaxis regimen were almost the same between the BMT/PBSCT and CBT recipients. Overall rates of high-risk patients were 62% for BMT/PBSCT recipients and 57% for CBT recipients ($P = .57$). On the other hand, there were significant differences in the following variables (Table 1). Patients receiving CBT had lower body weight and received transplants in a later calendar year. Sixty-seven percent of BMT/PBSCT recipients were administered a conditioning regimen without CY, and 91% of CBT recipients were administered a conditioning regimen with CY. The 6 possible matches between the recipient and the donor were scored serologically for HLA-A and HLA-B and genetically for DRB1 alleles, and the results showed 54 (76%) matched grafts in BMT/PBSCT recipients and no complete matches in CBT recipients. Details of HLA disparities between CBT recipients and grafts are described in the footnote to Table 1. Although the number of leukocytes for CBT recipients was 1 log lower than in BMT/PBSCT recipients, 93 of 100 cord blood grafts contained more than 2.0×10^7 cells/kg. The median number of $CD34^+$ progenitor cells was 0.93×10^5/kg (range, 0.15×10^5 to 8.97×10^5/kg) before freezing of cord blood grafts.

Engraftment, hematopoietic recovery, GVHD, and length of hospitalization

Four patients (4%) died within 28 days of cord blood transplantation, and primary graft failure occurred in 5 of the surviving 96 in the CBT recipient group. There was one early death (2%) on day 7 due to multiple organ failure in the BMT/PBSCT recipient group, but no patients had primary graft failure.

Patients receiving CBT had significantly slow neutrophil and platelet recovery in multivariate analysis (Table 2), in contrast with almost comparable recoveries in hematopoietic engraftment with longer-term follow-up. The overall myeloid engraftment rates on day 60 were 91% (95% confidence interval [CI], 85% to 97%) for CBT recipients and 96% (95% CI, 91% to 100%) for BMT/PBSCT recipients. Platelet counts of more than 2×10^9/L on day 100 were 85% (95% CI, 78% to 92%) and 94% (95% CI, 89% to 100%), and platelet counts of more than 5×10^9/L on day 180 were 83% (95% CI, 75% to 91%) and 92% (95% CI, 85% to 98%) for CBT and BMT/PBSCT recipients, respectively. One hundred percent of donor chimerisms were confirmed in all recipients after hematopoietic recovery by the techniques described in "Patients, materials, and methods."

More than 90% of patients in both groups received CsA plus a short-term MTX regimen as GVHD prophylaxis. The tapering rate of immunosuppressant drugs differed among individual patients due to variations in GVHD severity, renal function, and primary disease risk, although the protocol was assigned as described previously.[6] Consequently, the rate of decreasing immunosuppressants and discontinuation for CBT recipients was faster than those for BMT/PBSCT recipients (Table 3). The cumulative incidence of grades II to IV acute GVHD in both groups was almost equivalent (Figure 1A). On the other hand, despite the rapid tapering of prophylactic drugs for GVHD and the high degree of HLA disparity among CBT recipients, the cumulative incidence of grades III and IV acute GVHD was significantly lower in multivariate analysis (hazard ratio: 0.38; 95% CI: 0.15 to 0.95; $P = .04$; Table 2 and Figure 1B). The cumulative incidence of requiring steroids for treating acute GVHD among CBT recipients was significantly lower than among BMT/PBSCT recipients (hazard ratio: 0.25; 95% CI: 0.13 to 0.50; $P < .01$; Table 2 and Figure 1C).

Chronic GVHD affected 73 of 82 CBT and 49 of 55 BMT/PBSCT recipients surviving more than 100 days. Twenty-three CBT and 30 BMT/PBSCT recipients developed extensive GVHD. The incidence of overall chronic GVHD in CBT recipients tended to be higher than that in BMT/PBSCT recipients (Table 2; Figure 1D); however, the cumulative incidence of extensive-type GVHD among CBT recipients was significantly lower than that among recipients using grafts from related donors (hazard ratio: 0.49; 95% CI: 0.29 to 0.85; $P = .01$; Table 2 and Figure 1E).

Eighty-three CBT recipients and 59 BMT or PBST recipients were discharged from the hospital. The median number of days of hospitalization for CBT recipients was 121 and tended to be longer than that for BMT/PBSCT recipients, which was 89 days after transplantation (hazard ratio: 0.73; 95% CI: 0.450 to 1.06; $P = .10$; Table 2).

Table 2. The results of multivariate analysis of time to engraftment, GVHD, and length of hospitalization

	BMT or PBSCT recipient	CBT recipient	Hazard ratio (95% CI)*	P
Recipients, n	71	100	—	—
Absolute neutrophil count higher than 0.5 × 10^9/L			0.14 (0.10-0.22)	< .01
No. of patients to achieve (%)	69 (97)	91 (91)	—	—
Median	17	22	—	—
Range	10-35	16-46	—	—
Platelet count higher than 20 × 10^9/L			0.19 (0.13-0.28)	< .01
No. of patients to achieve (%)	68 (96)	85 (85)	—	—
Median	22.5	40	—	—
Range	12-122	13-99	—	—
Platelet count higher than 50 × 10^9/L			0.25 (0.17-0.36)	< .01
No. of patients to achieve (%)	67 (94)	84 (84)	—	—
Median	27	46	—	—
Range	13-453	25-263	—	—
Acute GVHD			—	—
No. of evaluable patients†	66	85	—	—
Grade, no. (%)			—	—
0	6 (9)	4 (5)	—	—
I	24 (36)	30 (35)	—	—
II	23 (35)	45 (53)	—	—
III	8 (12)	4 (5)	—	—
IV	5 (8)	2 (2)	—	—
II-IV	36	51	1.09 (0.71-1.68)	.69
III-IV	13	6	0.38 (0.15-0.95)	.04
Patients treated with steroids	28	18	0.25 (0.13-0.50)	< .01
Chronic GVHD			—	—
No. of evaluable patients‡	55	82	—	—
Limited + extensive	49	73	1.43 (0.95-2.14)	.09
Extensive	30	23	0.49 (0.29-0.85)	.01
Length of hospitalization			0.73 (0.50-1.06)	.10
No. of evaluable patients	59	83	—	—
Median	89	121	—	—
Range	39-355	58-608	—	—

CI indicates confidence interval; GVHD, graft-versus-host disease; and —, not applicable.

*The hazard ratio is for cord blood transplantation compared with bone marrow or peripheral blood stem-cell transplantation.

†Acute GVHD was evaluated in patients surviving 21 days or longer after transplantation with evidence of engraftment.

‡Chronic GVHD disease was evaluated in patients surviving 100 days or longer after transplantation with engraftment.

In CBT recipients, HLA disparities did not have any significant effects on engraftment, hematopoietic recovery, GVHD, or length of hospitalization.

TRM, relapse, and DFS

The respective 100-day and 1-year cumulative incidences of TRM were 8% (95% CI, 3% to 14%) and 9% (95% CI, 3% to 15%) among CBT recipients, and 4% (95% CI, 0 to 9%) and 13% (95% CI, 4% to 21%) among BMT/PBSCT recipients (Figure 2A). Higher age and higher risk of disease had significant impacts, as shown in Table 4, but the source of graft did not. Higher risk of disease and the diagnosis of ALL were significantly poor factors for relapse. Higher risk of disease was also a significant risk factor on DFS results in the multivariate analysis, as shown in Table 4.

There was no apparent difference between the risk of relapse and DFS in both groups in multivariate analysis (Table 4). The 3-year cumulative incidence of relapse was 17% (95% CI, 9% to 25%) in CBT recipients and 26% (95% CI, 15% to 37%) in BMT/PBSCT recipients (Figure 2B). The 3-year probabilities of Kaplan-Meier–estimated DFS were 70% (95% CI, 61% to 80%) after cord blood transplantation and 60% (95% CI, 49% to 72%) after bone marrow transplantation/peripheral blood stem-cell transplantation (Figure 2C). Because the ratio of high-risk disease in the BMT/PBSCT recipient group (62%) was slightly higher than in the CBT recipient group (57%, $P = .57$), we compared DFS rates of both groups for each disease risk. DFS of both groups was also equivalent in standard-risk patients and high-risk patients. The 3-year probabilities of DFS were 93% (95% CI, 85% to 100%) after cord blood transplantation and 85% (95% CI, 71% to 99%) after bone marrow transplantation/peripheral blood stem-cell transplantation in recipients in the standard-risk disease category (Figure 2D), and those in recipients in the high-risk disease category were 56% (95% CI, 42% to 70%) after cord blood transplantation and 45% (95% CI, 30%

Table 3. The results of multivariate analysis of tapering rate of immunosuppressants after transplantation

	Median d (no. of patients to achieve, range)			
	BMT or PBSCT recipient	CBT recipient	Hazard ratio (95% CI)	P
Decreased to 50% dose	47 (57, 7-1910)	44 (83, 7-149)	1.49 (1.05-2.12)	.03
Termination	383 (37, 51-1910)	188.5 (58, 50-1197)	4.10 (2.17-7.72)	< .01

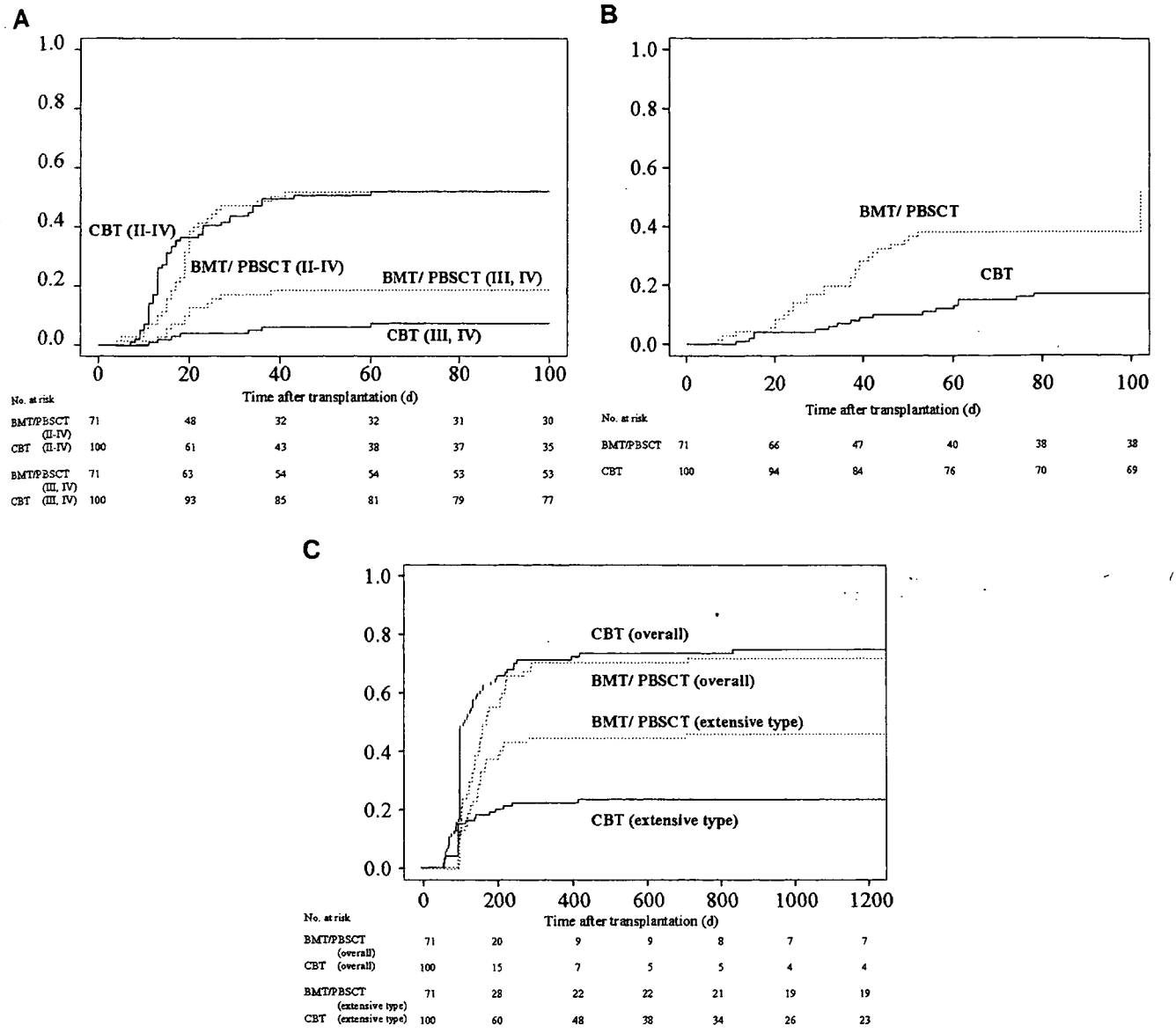

Figure 1. Cumulative incidences of acute and chronic GVHD after transplantation and kinetics of immunosuppressant use after transplantation. (A) Cumulative incidence of acute GVHD. The values of grades II to IV acute GVDH on day 100 were 52% (95% CI, 42% to 62%) for CBT and 52% (95% CI, 40% to 64%) for BMT/PBSCT recipients ($P = .69$). The values of grades III and IV acute GVDH on day 100 were 7% (95% CI, 2% to 13%) for CBT and 19% (95% CI, 19% to 28%) for BMT/PBSCT recipients ($P = .04$). (B) The cumulative incidence of requiring steroid therapy in patients after cord blood transplantation and bone marrow transplantation/peripheral blood stem-cell transplantation. The values on day 100 were 17% (95% CI, 10% to 24%) for CBT and 38% (95% CI, 27% to 49%) for BMT/PBSCT recipients ($P < .01$). (C) Cumulative incidence of chronic GVHD in patients surviving more than 100 days. The values for overall chronic GVHD were 71% (95% CI, 62% to 80%) at 1 year and 74% (95% CI, 40% to 64%) at 3 years after cord blood transplantation, in contrast to 68% (95% CI, 56% to 79%) at 1 year and 69% (95% CI, 58% to 80%) at 3 years after bone marrow transplantation/peripheral blood stem-cell transplantation ($P = .09$). The values of the cumulative incidence of extensive-type GVDH were 22% (95% CI, 14% to 30%) at 1 year and 25% (95% CI, 15% to 32%) at 3 years after cord blood transplantation, in contrast to 44% (95% CI, 32% to 55%) at 1 year and 45% (95% CI, 33% to 57%) at 3 years after bone marrow transplantation/peripheral blood stem-cell transplantation ($P = .01$).

to 60%) after bone marrow transplantation/peripheral blood stem-cell transplantation.

The proportion of causes of death was equivalent between CBT and BMT recipients (Table 5): almost 10% of deaths in both groups were GVHD related. The major cause of death in both recipient groups was relapse.

Discussion

Recently, 2 registration-based retrospective studies comparing the results of unrelated cord blood transplantation and unrelated bone marrow transplantation in adults have been published.[10,11] The investigators concluded that unrelated cord blood is an acceptable alternative source of hematopoietic stem cells for adults with acute leukemia who do not have an HLA-matched marrow donor. We have also reported on a comparative analysis of CBT versus BMT from unrelated donors for adult patients at our institute.[6] The overall results for CBT recipients were better than for BMT recipients in terms of GVHD, TRM, and DFS; moreover, our results for CBT recipients were better than those reported in European or US studies.

In this analysis, we compared updated results of unrelated cord blood transplantation with those of related transplantations in our hospital and demonstrated an equivalent safety and efficacy

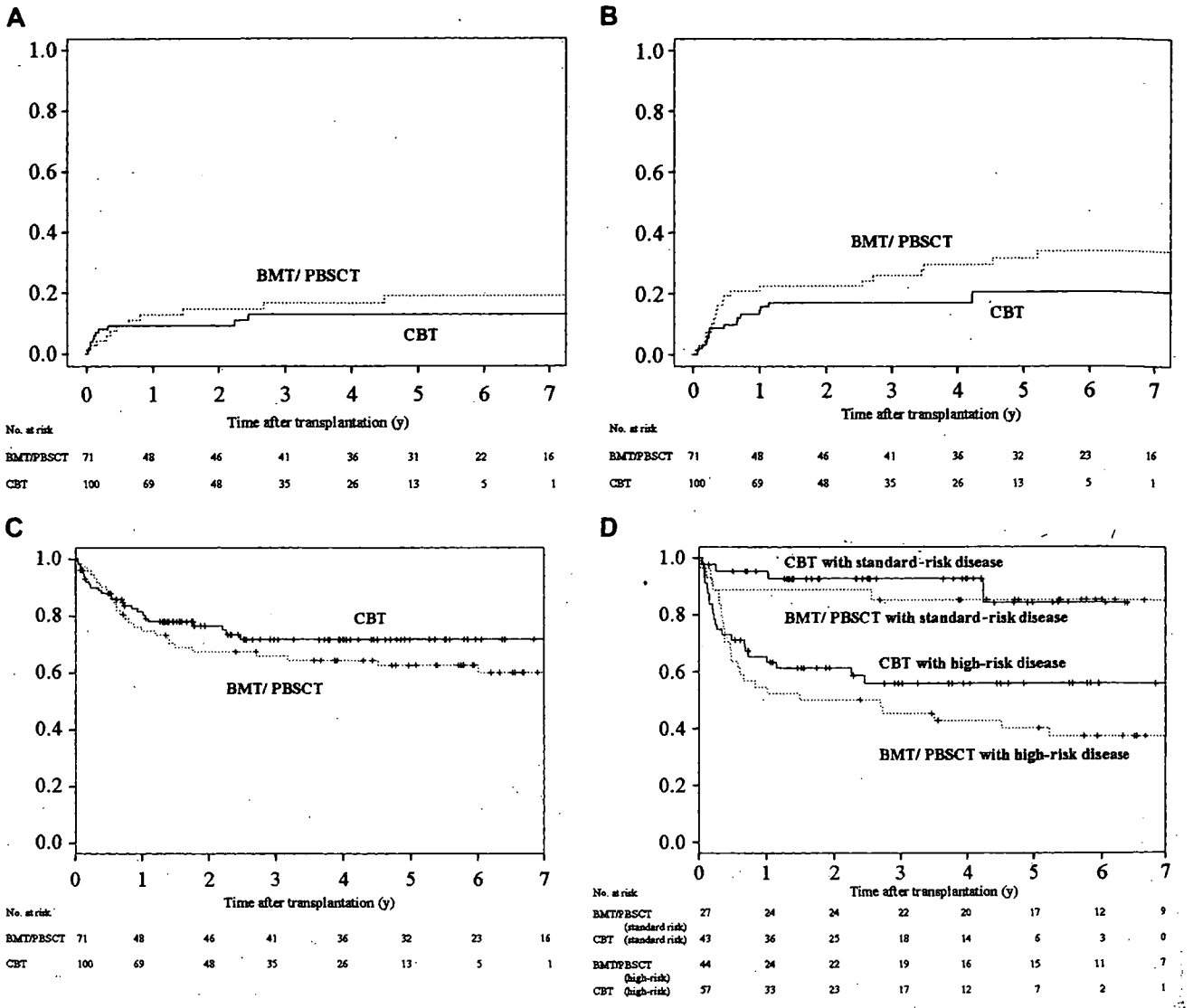

Figure 2. Outcomes among CBT and BMT/PBSCT recipients. (A) The 1-year and 3-year cumulative incidences of TRM were 8% (95% CI, 3% to 14%) and 9% (95% CI, 3% to 15%) among CBT recipients, respectively, in contrast to 4% (95% CI, 0 to 9%) and 13% (95% CI, 4% to 21%) among BMT/PBSCT recipients, respectively. The differences between the 2 groups were not significant ($P = .13$). (B) The 3-year cumulative incidences of relapse among recipients were 17% (95% CI, 9% to 25%) after cord blood transplantation and 26% (95% CI, 15% to 37%) after bone marrow transplantation/peripheral blood stem-cell transplantation. The differences between the 2 groups were not significant ($P = .34$). (C) The 3-year Kaplan-Meier estimate of DFS was 70% (95% CI, 61% to 80%) after cord blood transplantation and 60% (95% CI, 49% to 72%) after bone marrow transplantation/peripheral blood stem-cell transplantation. The differences between the 2 groups were not significant ($P = .26$). (D) The 3-year Kaplan-Meier estimate of DFS in patients with standard-risk disease was 93% (95% CI, 85% to 100%) after cord blood transplantation and 85% (95% CI, 71% to 99%) after bone marrow transplantation/peripheral blood stem-cell transplantation. The differences between the 2 groups were not significant by nonadjusted comparison ($P = .72$). The 3-year Kaplan-Meier estimate of DFS in patients with high-risk disease was 56% (95% CI, 42% to 70%) after cord blood transplantation and 45% (95% CI, 30% to 60%) after bone marrow transplantation/peripheral blood stem-cell transplantation. The differences between the 2 groups were not significant by nonadjusted comparison ($P = .26$).

between both. Hematologic recovery was slower after cord blood transplantation, although the overall engraftment rates were not significantly different, with more than 80% of patients in both groups achieving myeloid and platelet engraftment. Incidences of severe acute GVHD and extensive-type chronic GVHD were significantly lower in CBT recipients.

Patients receiving related grafts were treated at earlier dates, whereas CBT recipients were treated more recently. We used almost the same supportive care during the period for both recipients of BMT/PBSCT and CBT. On the other hand, with the anticipated improved survival rate overall in allogeneic transplantation patients over time, we thought this factor of treatment dates may have in part contributed to the equivalent survival observed in patients receiving related versus cord blood allogeneic grafts. However, this factor did not affect any clinical results in our multivariate analysis.

In the multivariate analysis, older age and high risk of disease had significant impacts in terms of TRM. High risk of disease was also a significant risk factor for relapse and DFS. The diagnosis of ALL was another significantly poor indicator of relapse. However, there was no apparent difference in the risks of TRM, relapse, and rate of DFS between the CBT and BMT/PBSCT recipient groups. DFS in both groups was also comparable among standard-risk and high-risk groups.

We speculated on several reasons for our favorable results in CBT recipients. One of the reasons might be the availability of grafts containing sufficient numbers of cells and because Japanese body size is relatively small. In fact, there were only 7 patients who received cord blood grafts containing less than 2.0×10^7 nucleated cells/kg body weight among our 100 CBT recipients. Secondly, Japanese patients might have some advantages in the setting of HLA-mismatched transplantation due to HLA or non-HLA immune

genetics.[7-9,18] In particular, there is mounting evidence indicating that polymorphisms in non-HLA immune mediators and host defense genes, such as tumor necrosis factor, interleukin-10, or their receptor genes, could affect the severity of GVHD.[19-21] The immunogenetics in Japanese[7-9,22] may also have contributed to the favorable results in related stem-cell transplantation[23,24] and unrelated bone marrow transplantation compared with reports from Western countries. This racial advantage might be significantly observed in the setting of allogeneic transplantation using HLA-mismatched grafts such as cord blood transplantation. Thirdly, the preparative conditioning and GVHD prophylaxis regimens used in our study might also have been favorable factors.

In addition to the just-mentioned reasons, the timing of transplantation is a very important factor relating to clinical results. The quick availability of cord blood as a stem-cell source is thought to be one of the most important advantages compared with unrelated bone marrow grafts.[6,25] If the patient was eligible for allogeneic transplantation but had no related donor, we generally selected a cord blood graft first, rather than waiting for the results of an unrelated marrow donor search. In fact, duration from diagnosis to transplantation was almost the same between the BMT/PBSCT and CBT recipients in the study. This might be one of the reasons for our favorable results in adult CBT recipients, especially in terms of TRM, compared with most previously published studies. The earlier timing contributes to better clinical outcomes in both patients with stable or advanced-stage disease at transplantation because disease progression can be prevented and accumulation of chemotherapy-induced tissue toxicity can be decreased. The rapid preparation of grafts may also increase the likelihood of achieving successful transplantation in patients.

Table 4. Multivariate analysis of factors associated with TRM, relapse, and DFS

Outcome/variable	Hazard ratio (95% CI)	P
TRM		
Age		.03
45 y or older	2.66 (1.12-6.30)	
Younger than 45 y	1.0	
Disease status		.01
High-risk disease	4.91 (1.43-16.8)	
Standard risk	1.0	
Graft source		.13
CBT	0.49 (0.19-1.24)	
BMT/PBSCT	1.0	
Relapse		
Disease status		< .01
High-risk disease	5.00 (2.07-12.1)	
Standard risk	1.0	
Diagnosis of primary disease		.03
ALL	2.37 (1.20-4.70)	
Other than ALL	1.0	
Graft source		.34
CBT	0.72 (0.37-1.41)	
BMT/PBSCT	1.0	
DFS		
Disease status		< .01
High-risk disease	5.37 (2.54-11.4)	
Standard risk	1.0	
Graft source		.26
CBT	0.74 (0.44-1.25)	
BMT/PBSCT	1.0	

TRM indicates transplant-related mortality; ALL, acute lymphoblastic leukemia; and DFS, disease-free survival.

Table 5. Cause of death

	BMT or PBSCT recipient, no. (%)	CBT recipient, no. (%)
Total	26	25
Relapse/refractory disease	13 (50)	14 (56)
Infection	7 (27)	4 (16)
GVHD with or without infection	3 (12)	3 (12)
Organ failure with or without infection	3 (12)	4 (16)

GVHD indicates graft-versus-host disease.

Once a patient was considered eligible for allogeneic transplantation and did not have a related donor, we performed cord blood transplantation at the same timing as bone marrow transplantation/peripheral blood stem-cell transplantation from a related donor. On the other hand, in most institutes, including those in Japan, a cord blood graft was not selected as a primary graft for patients who did not have a family donor, which might be a reason for the different clinical results for cord blood transplantation among centers in Japan.[26]

In this analysis, we compared the clinical outcomes of CBT from unrelated donors with BMT/PBSCT from related donors including HLA-matched siblings and also HLA-mismatched relatives, the latter 2 types of donors being quickly available. We took this approach because HLA closely-matched relatives were previously considered as acceptable donors in some clinical settings in patients without HLA-matched sibling donors.[24,27-29]

Our clinical results suggest that cord blood from unrelated donors could be as safe and effective a stem-cell source as bone marrow or mobilized peripheral blood from related donors for adult patients when used as a primary unrelated stem-cell source.

Acknowledgments

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Authorship

Contribution: S.T. and S.A. designed the study; J.O., A.T., T.K., N.T., K.F., M.U., K.T., T.I., and A.T. performed patients' care; M.O.M. performed data management; T.Y. analyzed data statistically; and S.T. wrote the paper.

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JTE-607, a multiple cytokine production inhibitor, ameliorates disease in a SCID mouse xenograft acute myeloid leukemia model

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Objective. **Accumulating findings suggest that in acute myeloid leukemia (AML) patients, proinflammatory cytokines and growth factors play important roles in the proliferation and survival of AML cells in an autocrine and paracrine manner, leading to deterioration of AML. JTE-607 is a multiple cytokine inhibitor that potently suppresses production of proinflammatory cytokines. In the present study, we investigated the potency of JTE-607 as an antileukemic agent by exploiting a SCID mouse acute leukemia model.**

Methods. **SCID mice injected with anti-asialo-GM1 antibody were exposed to sublethal total-body irradiation at a dose of 3 Gy and then inoculated intravenously with AML cells. JTE-607 was administered using osmotic minipumps. The effects of JTE-607 on mouse survival time, human interleukin (IL)-8 levels in mouse plasma, and proportion of human $CD45^+$ cells in the bone marrow were studied.**

Results. **The survival time of the mice was strictly dependent on the number of U-937 cells proliferating in vivo. Administration of JTE-607 during the initial 7 days significantly prolonged survival of the mice, suggesting killing activity of JTE-607 against AML cells in vivo. Delayed administration of JTE-607 also prolonged the survival of mice bearing established leukemia with an effect comparable to the maximum tolerable dose of cytarabine. Flow cytometer analysis of bone marrow cells revealed decreased number of human $CD45^+$ cells. Human IL-8 level was also reduced by JTE-607.**

Conclusion. **Our results indicate that JTE-607 has potential to be a new class of antileukemic drug that exerts inhibitory activities against both the proliferation and proinflammatory cytokine production of AML cells.** © **2006 International Society for Experimental Hematology. Published by Elsevier Inc.**

Acute myeloid leukemia (AML) is an aggressive disorder characterized by expansion and accumulation of immature leukemia cells arrested at various stages in the bone marrow [1]. Recent advances in chemotherapy of AML have improved clinical outcome, inducing complete remission in 70 to 80% of AML patients during initial treatment [2,3]. However, in spite of current intensive chemotherapy strategies, disease-free survival remains as low as 30 to 50%, mainly because of relapse after treatment [1–3]. In addition, although allogenic bone marrow transplantation is the most efficacious against relapse, many patients cannot tolerate the high burden of this aggressive therapy [4]. Therefore, novel therapeutic approaches are still required to prevent recurrence and provide beneficial treatment even after relapse.

It has been thought that growth, survival, and resistance to antileukemic agents of AML cells are promoted and sustained by close interaction with stromal cells in the bone marrow microenvironment through local production of cytokines and growth factors (e.g., VEGF, IL-8, and GM-CSF) in an autocrine and paracrine manner [5–10]. In fact, it has been reported that the production of growth factors from AML blasts is upregulated [5,9,11–14] and a strong relationship exists between the circulating levels of growth factors and prognosis of AML [11,15–17]. In addition, increased production of proinflammatory cytokines by leukemic blasts promotes bone marrow neoangiogenesis in a paracrine manner and increased angiogenesis has been demonstrated in the bone marrow of patients with AML

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[18,19]. It was recently demonstrated that thalidomide exerts anti-angiogenesis effect by reducing the release of growth factor by endothelial cells [20] and its clinical efficacy against hematopoietic malignancies, including multiple myeloma (MM), myelodysplastic syndromes (MDS), and AML has been demonstrated [21–24]. Thus, depression of the cytokine / growth factor network is thought to be a new approach to overcome the limitations of current chemotherapy for AML.

JTE-607 [(-)-Ethyl-N-{3,5-dichloro-2-hydroxy-4-[2-(4-methylpiperazin-1-yl)ethoxy]benzoyl}-L-phenylalaninate dihydrochloride] (Fig. 1) was originally discovered as a multiple cytokine inhibitor that broadly suppresses production of proinflammatory cytokines such as IL-1β, IL-6, IL-8, GM-CSF, and TNF-α from human peripheral blood mononuclear cells (PBMCs) stimulated with lipopolysaccharide [25]. In in vivo studies, JTE-607 improved the survival rate in a cecal ligation and puncture-induced mouse septic shock model [26] and showed inhibitory effect on the burn insult–induced mouse lung injury [27]. Because of the presumed importance of cytokine in the pathology of AML, we examined the effect of JTE-607 on AML cell lines and found that JTE-607 suppressed not only spontaneous cytokine production but also proliferation of AML cells in vitro. The cells exposed to JTE-607 showed cell-cycle arrest at S phase and subsequently underwent apoptosis, accompanied by the decrease in c-Myc and the increase in $p21^{waf1/cip1}$ protein levels (manuscript in preparation).

In the present study, we exploited an acute leukemia model established by engraftment of U-937 cells in preconditioned SCID mice, in which the survival of mice was directly correlated with the number of leukemia cells proliferating in vivo. Administration of JTE-607 using osmotic minipumps reduced the number of leukemic cells in the bone marrow and significantly prolonged the mouse survival even after the development of leukemia. Moreover, human interleukin (IL)-8 level in mouse plasma was reduced by injection of JTE-607, indicating both proliferation and cytokine production of AML cells was effectively blocked. Thus, we conclude that JTE-607 is a promising candidate for an antileukemic drug that may bring about a new approach in the therapy of AML.

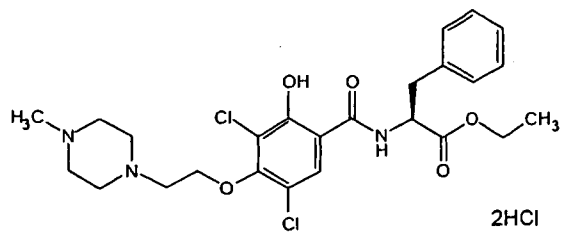

Figure 1. Chemical structure of JTE-607 [(-)-Ethyl-N-{3,5-dichloro-2-hydroxy-4-[2-(4-methylpiperazin-1-yl)ethoxy]benzoyl}-L-phenylalaninate dihydrochloride.

Materials and methods

AML cell lines

Human myeloid leukemia cell lines U-937, HL-60, and THP-1 were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). The cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), L-glutamine (2 mmol/L), penicillin (100 Units/mL), and streptomycin (100 μg/mL), and maintained at 37°C in a humidified atmosphere that contained 5% CO_2.

Reagents

JTE-607 was chemically synthesized at Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Osaka, Japan). Cytosine-1-β-D(+)-arabinofuranoside (cytarabine) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). For in vitro studies, these drugs were dissolved in dimethylsulfoxide (DMSO) and diluted with cell culture medium to 1% DMSO. For in vivo studies, JTE-607 was dissolved in 30% hydroxypropyl β-cyclodextrin (HP-β-CD) aqueous solution. Cytarabine was dissolved in saline.

In vitro cell proliferation assay

AML cells were seeded in 96-well flat-bottom microplates at 5×10^2 to 3×10^3 cells/well and incubated in the presence of various concentrations of JTE-607 or cytarabine at 37°C for 3 days (final DMSO concentration: 0.1%). The cells were pulsed with 9.25 kBq/well of [^{3}H]-thymidine during the last 6 hours of culture, and [^{3}H]-thymidine incorporation was determined by liquid scintillation counting. All measurements were performed in triplicate. The 50% inhibitory concentration (IC_{50}) value was calculated in a semilogarithmic proportional manner from the two points enclosing 50% inhibition.

In vitro IL-8 production assay

U-937 cells were seeded in 96-well flat-bottom microplates at 2×10^5 cells/well and incubated with various concentrations of JTE-607 in the presence or absence of LPS (10 μg/mL) at 37°C for 24 hours (final DMSO concentration: 0.1%). Human IL-8 concentrations in the supernatants were measured using a specific ELISA kit (R&D systems, Minneapolis, MN, USA). All measurements were performed in duplicate.

SCID mouse xenograft acute leukemia model

Eight- to 9-week-old female SCID (Fox Chase C.B-17/Icr-scidJcl) mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and housed under specific pathogen-free conditions. Sterile food and water was given ad libitum. Mice were injected intraperitoneally with rabbit anti-asialo-GM1 antibody to deplete natural killer cells. Twenty-four hours after the antibody injection, the mice were exposed to sublethal total-body irradiation at a dose of 3 Gy using a soft x-ray ionization chamber (M-150WE; Softex, Tokyo, Japan) and then inoculated intravenously with AML cells in 200 μL of Hank's Balanced Salt Solution (day 0). For early treatment with JTE-607, Alzet osmotic minipumps (model 2001, Durect Corp., CA, USA) filled with JTE-607 or vehicle solution were implanted under dorsal skin of mice immediately after the U-937 inoculation. In accordance with the pump specifications, concentrations of JTE-607 were adjusted to dosages of 0.2, 0.6, and 1.8 mg/animal/day (10, 30, and 90 mg/kg/day). The nominal duration of pumping was 7 days. For delayed treatment,

minipumps filled with JTE-607 solution for which concentration was adjusted to dosages of 0.6 and 1.8 mg/animal/day (30 and 90 mg/kg/day) were implanted on day 12. Cytarabine was subcutaneously injected at dosages of 3, 30, and 300 mg/kg/day, once a day for 4 days from day 12. General condition, body weight, and survival of the mice were monitored and mean survival days were compared as indication of antileukemic effects. All procedures for animals were reviewed and approved by the Animal Care and Management Committee of Central Pharmaceutical Research Institute, Japan Tobacco Inc.

Detection of human IL-8 in plasma and CD45$^+$ cells in bone marrow

The SCID mice engrafted with U-937 cell were treated with JTE-607 (90 mg/kg/day) for the initial 7 days as described above (early treatment). On day 18, heparinized plasma samples were collected and used for measurement of human IL-8 concentration using a human IL-8 ultra-sensitive ELISA kit (R&D Systems, Minneapolis, MN, USA) that has no cross-reactivity with mouse IL-8. Bone marrow cells of the mice were then recovered by flushing mouse femurs and tibias with ice-cold Hank's Balanced Salt Solution. After removing erythrocytes, the cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD45 (BD Biosciences Pharmingen, San Jose, CA, USA) or isotype control antibody and analyzed by a FACScan flow cytometer (Becton-Dickinson, San Jose, CA, USA). The percentage of human CD45$^+$ cells (U-937 cells) in total bone marrow cells was determined by data analysis using CellQuest software (Becton-Dickinson, San Jose, CA, USA).

In the bolus treatment study, the SCID mice engrafted with U-937 cell were subcutaneously injected once with JTE-607 (100 mg/kg) or vehicle solution on day 18. Three hours after the injection, blood samples and bone marrow cells were collected and analyzed in the same way.

Statistical analysis

Statistical significances between vehicle-treated control group and drug administration groups were determined using the Kaplan-Meier method and the log-rank test for the survival ratio (%) and the Mann-Whitney *U* test for the mean survival day (MSD) using SAS software (Ver. 8.2; SAS Institute Japan, Tokyo, Japan), respectively. Statistical analysis for CD45$^+$ cell in bone marrow and plasma IL-8 level were performed by Student's *t*-test. A p value of less than 0.05 was considered to be statistically significant.

Results

Inhibitory activity of JTE-607 on the in vitro proliferation of AML cell lines

In the beginning, we examined the effect of JTE-607 on the spontaneous production of proinflammatory cytokines from AML cell lines, and found that both mRNA levels in the cells and protein levels in the culture supernatant of IL-6 and IL-8 were significantly suppressed by JTE-607 (e.g., % inhibition on IL-6 and IL-8 production from HL-60 cells was 79.4% and 43.7% at the concentration of 1 μmol/L, respectively; manuscript in preparation). This observation led us to hypothesize that JTE-607 might interfere with the constitutively activated cellular responses that were ascribed to proliferation and survival of AML cells. In this line, we next examined the inhibitory effect of JTE-607 on the proliferation of human AML cell lines. As expected, JTE-607 inhibited the proliferation of U-937, HL-60, and THP-1 cells, with IC_{50} values of 0.029, 0.070, and 0.28 μmol/L, respectively (Table 1). The inhibitory activity of JTE-607 appeared to be most potent in U-937 cells, although the activity was 10-fold lower than that of cytarabine, whereas the activities of JTE-607 in HL-60 and THP-1 cells were approximately comparable to those of cytarabine.

Establishment of SCID mouse xenograft acute leukemia model

Engraftment of human leukemic cell lines in SCID mice has been reported to mimic human leukemia and provide suitable conditions for evaluation of antileukemic drugs [28,29]. To establish a well-controllable SCID mice xenograft leukemia model using AML cell lines, we first compared the ability of U-937, HL-60, and THP-1 cells to induce mortal leukemic disorders. The mice receiving anti-asialo GM1 antibody were exposed to sublethal total-body irradiation at a does of 3 Gy and subsequently injected with 1×10^6 AML cells via tail vein. All of the mice showed body weight loss, ruffled fur, and paralysis of hindlimb due to infiltration and expansion of AML cells in lumbar cord, then eventually died. However, duration to death in the mice engrafted with U-937 was much shorter than in the others: the MSD with U-937 was 20 ± 6.7, vs 47 ± 2.6 with HL-60 and 53 ± 1.7 with THP-1 (Fig. 2A). Since the short duration of disease development seemed to mimic aggressive leukemic burden in AML more closely, and as it is also suitable for investigation of in vivo efficacy of drugs, we selected U-937 cells and checked inoculation dose–dependent alteration in MSD. As shown in Figure 2B, the mice inoculated with graded doses of U-937 cell exhibited dose-dependent shortening of survival days, showing 38 ± 4.4, 26 ± 2.6, 22 ± 1.3, and 17 ± 1.2 days for 10^4, 10^5, 10^6, and 10^7 cells of U-937, respectively. A negative correlation between MSD and the number of

Table 1. Inhibitory effects of JTE-607 and cytarabine on proliferation of acute myeloid leukemia cell line in vitro

	IC_{50} (μmol/L)	
AML cell line	JTE-607	Cytarabine
U-937	0.029	0.0032
HL-60	0.070	0.014
THP-1	0.28	0.17

Leukemia cell lines were cultured in 96-well flat-bottom microplates with various concentrations of JTE-607 and cytarabine for 72 hours. The growth inhibitory activities were determined by [^{3}H]-thymidine incorporation assay. Results were shown as the mean of 50% inhibitory concentration (IC_{50}) of two independent experiments.

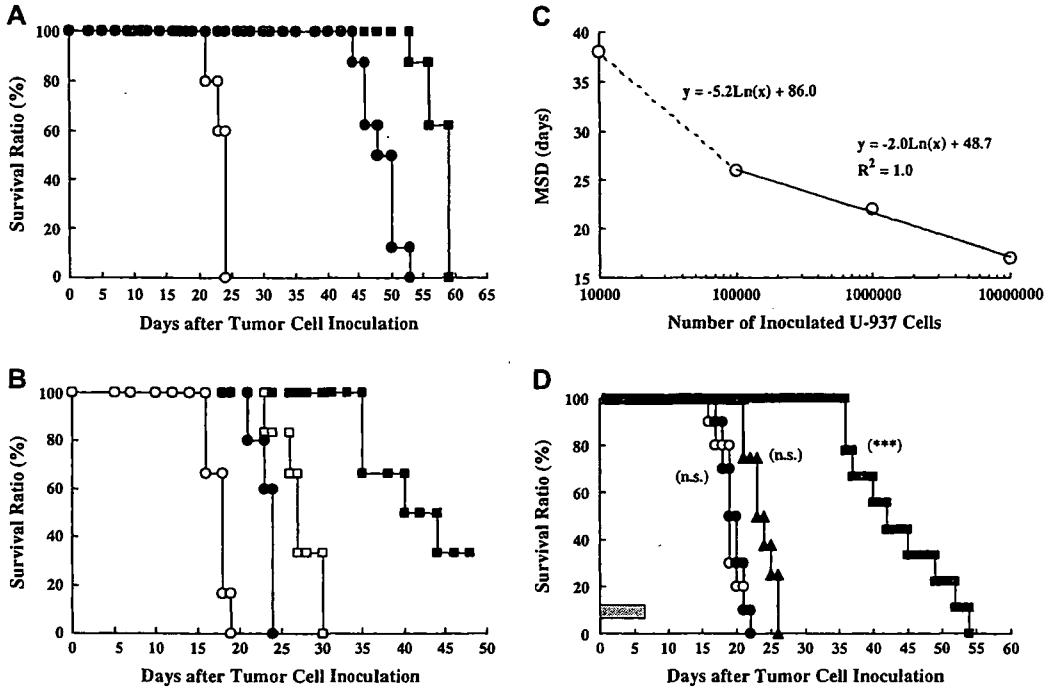

Figure 2. Establishment of a SCID mouse xenograft leukemia model and antileukemic activity of JTE-607. **(A)** Preconditioned SCID mice were inoculated intravenously with 1×10^6 cells of U-937 (○), HL-60 (●), or THP-1 cells (■) on day 0 (n = 8). Mice were observed daily for signs of leukemia and survival. **(B)** Preconditioned SCID mice were inoculated intravenously with 1×10^7 cells (○), 1×10^6 cells (●), 1×10^5 cells (■), or 1×10^4 cells (■) of U-937 on day 0 (n = 6). **(C)** The correlation between mean survival day (MSD) and number of U-937 inoculated. The linear correlation coefficient (R^2) was observed within a range of 10^5 to 10^7 cells. **(D)** Preconditioned SCID mice were inoculated intravenously with 1×10^6 cells of U-937 on day 0. JTE-607 was administered by continuous infusion using osmotic minipumps at dosages of 10 (●), 30 (▲), and 90 mg/kg/day (■) for 7 days, starting on day 0 (n = 10). Control mice (○) were given vehicle in the same way. Mice were observed daily for signs of leukemia and survival. Statistical analysis was performed by the Kaplan-Meier method and the log-rank test. ***: $p < 0.0001$ vs control group. n.s., not significant.

inoculated U-937 cells was observed at a dose of 10^4 to 10^7 cells, and a linear correlation coefficient (R2) was observed in the range of 10^5 to 10^7 cells (Fig. 1C). These results indicate the U-937 xenograft SCID mouse leukemia model examined here was a highly controllable model, which precisely reflects the number of leukemia cells proliferating in vivo and enables sensitive assessment of antileukemic activity of drugs.

Using the U-937 xenograft leukemia model, we first examined the effect of early treatment with JTE-607 on the MSD of mice. Because of its short half-life in vivo ($t_{1/2}\alpha$ = 0.1 hour in human), JTE-607 must be administered by intravenous infusion in clinical use. For this reason, in this study administration of JTE-607 was performed by continuous infusion using osmotic minipumps. Minipumps (nominal pumping duration: 7 days) filled with JTE-607 solution were placed under dorsal skin on day 0, immediately after U-937 inoculation. As a result, prolongation of the survival days was observed by treatment with JTE-607 at a dosage of 30 mg/kg/day and above, and was statistically significant at 90 mg/kg/day (Fig. 2D). The MSD for 10, 30, and 90 mg/kg/day was 20 ± 1.6, 24 ± 2.0, and 43 ± 6.9 days, respectively (Table 2). When proliferation of U-937 is cytostatically suppressed during the initial 7 days, the MSD is estimated to be 26 days from the tight correlation between cell number and MSD. The MSD for the 90 mg/kg/day group was much longer than this estimated MSD; therefore it was likely that JTE-607 exerted killing activity against U-937 cells in vivo.

Table 2. Summary of MSD (mean survival day) in SCID mice treated with JTE-607 and cytarabine

Treatment group	Dosage	MSD ± SD (days)	(p value)
Early treatment (osmotic minipump, S.C., day 0 ± 7, n = 10)			
Control		19 ± 1.7	
JTE-607	10 mg/kg/day	20 ± 1.6	(n.s.)
	30 mg/kg/day	24 ± 2.0	(n.s.)
	90 mg/kg/day	43 ± 6.9	($p < 0.001$)
Delayed treatment (osmotic minipump, S.C., day 12 ± 19, n = 10)			
Control		19 ± 1.5	
JTE-607	30 mg/kg/day	21 ± 1.2	(n.s.)
	90 mg/kg/day	26 ± 1.1	($p < 0.001$)
Delayed treatment (once a day, S.C., day12 ± 15, n = 10)			
Control		20 ± 1.8	
Cytarabine	3 mg/kg/day	22 ± 1.8	(n.s.)
	30 mg/kg/day	29 ± 2.3	($p < 0.001$)
	300 mg/kg/day	19 ± 1.4	(n.s.)

Statistical analysis was performed by Mann-Whitney U test. n.s., not significant vs control group; S.C., subcutaneously; SD, standard deviation.

In vivo therapeutic effect of JTE-607 and cytarabine (delayed treatment)

We next examined the therapeutic effect of JTE-607 on the established leukemia by delayed administration to predict its clinical potency. Continuous infusion of JTE-607 by osmotic minipumps (30 or 90 mg/kg/day, the nominal duration of pumping: 7 days) was started on day 12, at which the mice showed signs of leukemia (paralysis of hindlimb). In parallel, the other leukemia mice were subcutaneously injected with cytarabine at 3, 30, or 300 mg/kg/day for 4 consecutive days starting on day 12. JTE-607 showed its therapeutic potency at 90 mg/kg/day with significant prolongation of the mouse survival (Fig. 3A). The comparable effect was observed with cytarabine at a dosage of 30 mg/kg/day, and this was thought to be close to the maximum effect of cytarabine in this model, because dosage of 300 mg/kg/day cytarabine is apparently toxic (Fig. 3B). The efficacies of JTE-607 and cytarabine are summarized in Table 2, with MSD values.

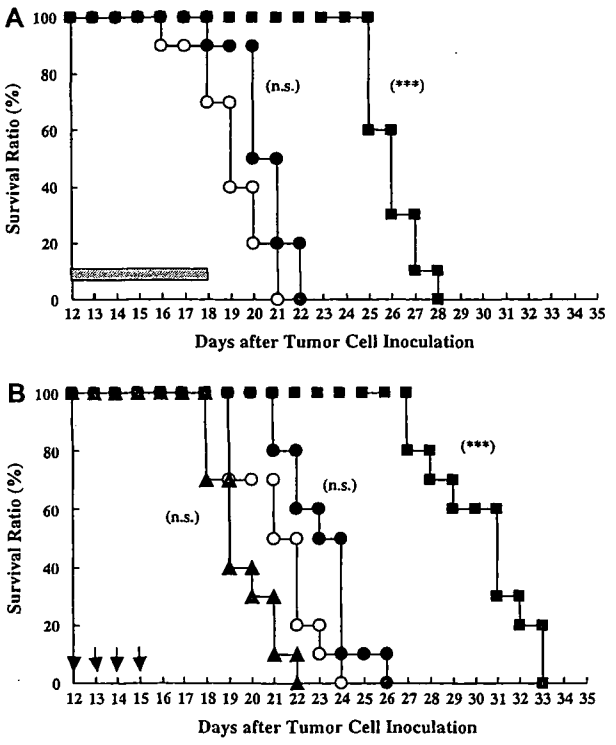

Figure 3. Therapeutic effects of JTE-607 and cytarabine in vivo. (A) Preconditioned SCID mice were inoculated intravenously with 1×10^6 cells of U-937 on day 0. JTE-607 was administered by continuous infusion using osmotic minipumps at dosages of 30 (●) and 90 mg/kg/day (■) for 7 days, starting on day 12 (n = 10). Control mice (○) were given vehicle in the same way. (B) The leukemia mice were subcutaneously injected with cytarabine at dosages of 3 (●), 30 (■), and 300 mg/kg/day (▲), once a day for 4 days from day 12 (n = 10). Control mice (○) were given vehicle in the same way. Mice were observed daily for signs of leukemia and survival. Statistical analysis was performed by the Kaplan-Meier method and the log-rank test. ***: $p < 0.0001$ vs corresponding control group. n.s., not significant.

Inhibitory effects on human IL-8 level in plasma and proportion of human CD45$^+$ cells in bone marrow

In the examination of bone marrow cells of the mice, it was revealed that human CD45$^+$ cells became detectable by flow-cytometer analysis following the appearance of signs of leukemia. The proportion of human CD45$^+$ cells reached 3 to 5% of total bone marrow cells just prior to death of mice (day 18). Human IL-8 also became detectable in the mouse plasma, indicating that lethal infiltration and expansion of U-937 cells in the mouse organs accompanied by massive cytokine production took place. To ascertain the diminishment of AML cells and inhibition of cytokine production by JTE-607, mice injected with U-937 cells were infused with 90 mg/kg/day of JTE-607 for the initial 7 days and sacrificed on day 18, then the proportion of human CD45$^+$ cells in bone marrow and human IL-8 levels in mice plasma were determined. For comparison, the same analysis was conducted with another group of mice that were administered 100 mg/kg of JTE-607 by bolus injection on day 18 and sacrificed 3 hours after the injection. The results showed that JTE-607 administered by infusion significantly reduced both the proportion of human CD45$^+$ cells and the human IL-8 level beyond the limits of detection (Fig. 4). The reduction of IL-8 level was also observed 3 hours after the bolus injection of JTE-607, whereas human CD45$^+$ cells in bone marrow were not altered by the ephemeral treatment (Fig. 4). These results confirm the antileukemic effect of JTE-607 that is exerted through a time-dependent process, and the fast response of AML cells to the activity of JTE-607 on cytokine production in vivo.

Inhibitory effects of JTE-607 on IL-8 production from U-937 cell in vitro

The appearance of human IL-8 in the mouse plasma suggests a large amount is produced from U-937 cells, but this cannot be accounted for by the low level spontaneous production in vitro. It is most likely that U-937 cells were activated in vivo and their cytokine production was upregulated. To examine alterations in the IL-8 production of U-937 cells and in the effect of JTE-607 on it, U-937 cells were exposed to JTE-607 for 24 hours with or without LPS stimulation. The IL-8 protein level in the culture supernatant was 11.9 pg/mL without stimulation, and was markedly increased by LPS to 198.5 pg/mL. JTE-607 inhibited the production of IL-8 concentration-dependently, regardless of LPS stimulation; however, the inhibitory activity against LPS-stimulated production was stronger than for spontaneous production (Fig. 5). These results are in good concordance with the remarkable reducing effect of JTE-607 on the plasma IL-8 level in vivo.

Discussion

AML is an aggressive disorder with disease-free survival of 30 to 50% even for patients treated with intensive

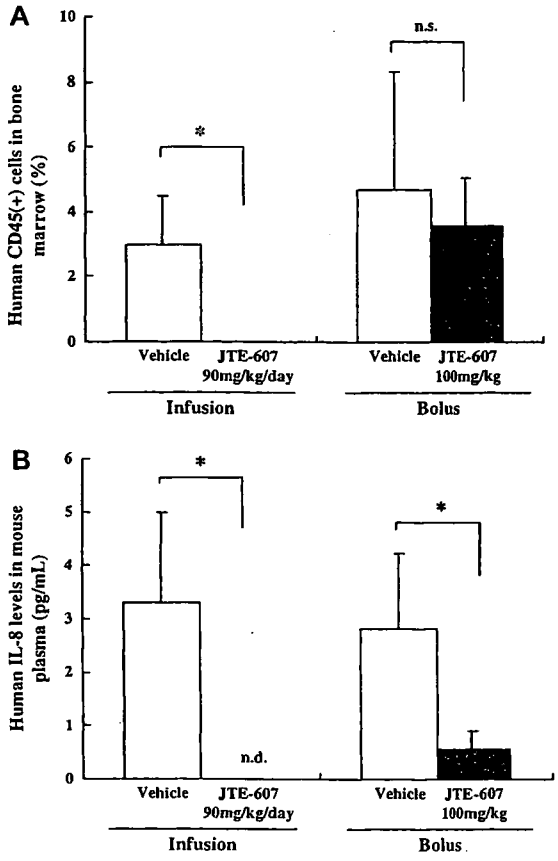

Figure 4. Effects of JTE-607 on human IL-8 level in plasma and proportion of human $CD45^+$ cells in the bone marrow of SCID mice inoculated with U-937 cells. (**A**) Preconditioned SCID mice were inoculated intravenously with 1×10^6 cells of U-937 on day 0. JTE-607 was administered by continuous infusion using osmotic minipumps at a dosage of 90 mg/kg/day for 7 days starting on day 0, or bolus injection at a dose of 100 mg/kg at 3 hours prior to blood collection on day18. Blood samples and bone marrow cells were collected on day18. (**A**) The proportion of human $CD45^+$ cells in the bone marrow cells was determined by flow-cytometry analysis after staining with FITC-labeled anti-human CD45 and isotype control antibody. Results are expressed as the mean ± standard error (s.e.) (n = 4–5). (**B**) The concentrations of human IL-8 in plasma were measured by ELISA assay. The detection limit of the assay was 0.5 pg/mL. Results are expressed as the mean ± s.e. (n = 4-5). Statistical analysis was performed using one-way ANOVA by Student's *t*-test *: $p < 0.05$ vs vehicle control group. n.s., not significant.

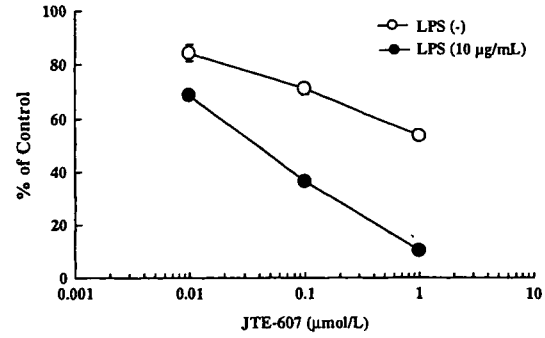

Figure 5. Inhibitory effect of JTE-607 on IL-8 production in U-937 cell in the presence or absence of LPS. U-937 cells (2×10^5) were cultured in 24-well flat-bottom microplates for 24 hours in the presence or absence of LPS (10 μg/mL) with indicated concentration of JTE-607 or vehicle. IL-8 concentrations in the culture supernatant were determined by ELISA. Results are expressed as the mean of % of vehicle control in two or three independent experiments.

conventional chemotherapy, because of relapses [1–3]. The crucial roles of inflammatory cytokines and growth factors in the autonomous proliferation of AML blasts are well documented [9]. The majority of leukemic blasts from AML patients produce several inflammatory cytokines and growth factors, such as IL-1, IL-3, IL-6, IL-8, VEGF, GM-CSF, G-CSF, and TNF-α at various levels [11,13,30,31], and a substantial portion of blasts exhibit autonomous proliferation by stimulation with these factors in vitro [7,30]. Inflammatory cytokines produced from AML cells can also stimulate production of growth factors and angiogenic factors from bone marrow stromal cells [5,8,32,33], which support proliferation of AML cells and induce angiogenesis in bone marrow [18,34]. The circulating levels of growth factor in patients with AML is markedly elevated at diagnosis, but decrease to the levels of the normal controls when patients are under chemotherapy or in complete remission [35]. In addition, several lines of evidence suggest that these autocrine and paracrine signalings confer antiapoptotic phenotype and resistance to conventional antileukemic agents in AML cells. In fact, beneficial effects in the clinical application of thalidomide, which inhibits growth factor production, suggest important roles of the autocrine and paracrine signalings in the pathology of hematopoietic malignancies including AML [20,24]. Thus, suppression of cytokine production from AML cells is expected to be a beneficial treatment for AML patients.

JTE-607 has several unique biological activity profiles. JTE-607 inhibits cytokine production from human PBMCs stimulated with LPS, TNF-α, and PMA, with IC_{50} values on the order of 1 nmol/L. The inhibitory activity of JTE-607 emerges as myeloid cell–specific, and no inhibition was observed in the cytokine production from LPS-stimulated fibroblasts or CD3/CD28-stimulated T cells [25]. On the basis of these characteristics, we first examined the effect of JTE-607 on human AML cell lines and found that JTE-607 significantly suppressed cytokine productions that were spontaneously upregulated, such as IL-6 and IL-8. Our work subsequently demonstrated that JTE-607 suppressed proliferation of AML cell lines and AML blasts in patients, by inducing cell-cycle arrest at S phase and apoptosis with accompanying downregulation of c-Myc and upregulation of p21 (manuscript in preparation). Furthermore, unlike conventional antileukemic drugs, the inhibitory effect of JTE-607 on CFU-GM of normal human bone marrow cells was 10 to 100 times weaker than those in the proliferation assay of AML cell lines. Western blot analysis revealed that spontaneous phosphorylation of p38 MAPK and MEK1/2 in U-937 cells was partially reduced

by JTE-607 (data not shown). Although the molecular target of JTE-607 has not yet been identified, these findings suggest that the target of JTE-607 is upstream of both MKK3/6-p38 and MEK1/2-ERK pathways. p38 MAPK is well known to be responsible for inflammatory cytokine production, and ERK is also responsible for cytokine production as well as cell proliferation [36,37]. Thus, in vitro and in vivo effects of JTE-607 are likely to represent concurrent inhibition of constitutively activated p38 and ERK pathways, and ERK inhibition promotes alteration of c-Myc and p21 levels, leading to cell-cycle arrest and apoptosis. It is also noteworthy that JTE-607 has ability to exert these effects without affecting growth of normal bone marrow cells or hematopoietic growth factor production from stromal cells, which are critical for maintenance and recovery of normal bone marrow hematopoiesis after conventional chemotherapy and radiotherapy. With respect to the presumed mechanism of action, sensitivity of AML cells to JTE-607 may be determined by importance of p38 and ERK pathways in cytokine production and autonomous proliferation. However, we have not conducted comparative analysis of MAPK activities in each cell line.

In the present study, we focused on clarifying whether JTE-607 could exert its unique activity on both proliferation and cytokine production in AML cells in vivo. To this end, we employed a SCID xenograft acute leukemia model in which preconditioned SCID mice were engrafted with U-937 cells intravenously. Among the AML cell lines tested, U-937 cells showed the most vigorous proliferation in the mice, which was thought to mimic aggressive leukemic burden in AML and to be suitable for investigation of efficacy of drugs. In addition, the strict correlation between MSD and number of U-937 cells inoculated indicates that MSD directly reflects the number of cells remaining and proliferating in vivo: therefore accurate and sensitive evaluation of antiproliferative effects of drugs can be performed by simple comparison of MSD. In this model, JTE-607 administered at 90 mg/kg/day by continuous infusion for the initial 7 days prolonged MSD to 43 days. Because the mice of control group receiving 10^6 cells showed an MSD of 19 days, if the effect of JTE-607 is cytostatic and the cells restart to grow following the end of administration, then the MSD should be prolonged 7 days and estimated to be 26 days (19 + 7 days). The MSD for the 90 mg/kg/day group was much longer than this estimated MSD, and the prolongation corresponds to a roughly 1/1000 reduction in number of U-937 cells, suggesting killing activity of JTE-607 against U-937 cells in vivo. This was further confirmed by showing the reduced proportion of human $CD45^+$ cells in bone marrow cells even 12 days after the completion of continuous infusion. Moreover, JTE-607 also prolonged the mouse survival in the delayed-treatment study that was conducted to predict the clinical potential, and the effect was comparable with that of cytarabine that was administered with the optimal regimen in this model. Therefore, we conclude that JTE-607 has a potential to exhibit clinically beneficial effect with at least similar extent to that of cytarabine by its antiproliferative, apoptosis-inducing activity.

Among cytokines and growth factors produced from U-937 cells, IL-8 could be detected in the mouse plasma over the detection limit of ELISA after the establishment of leukemia. IL-8 is known as a proangiogenic mediator and is produced by AML blasts at high levels in most patients [12,14]. Although the spontaneous production level of IL-8 is low in U-937 cells in vitro, the production can be ultimately upregulated by LPS stimulation. It is therefore conceivable that preconditioning of the mice caused LPS leakage from gastrointestinal tract and release of cross-reactive inflammatory cytokines from the mouse tissues, and with these proinflammatory stimuli, U-937 cells were activated to produce a large amount of IL-8. We consider that the conditions in this model correspond to the elevated plasma levels of cytokines and growth factors commonly observed in AML patients, which is most likely due to the activation of AML blasts by various stimuli in pathophysiological processes. JTE-607 showed significant inhibition against this upregulated production of IL-8 even after single administration, without affecting human $CD45^+$ cell proportion in the bone marrow cells. Indeed, the inhibitory effect of JTE-607 on IL-8 production was more potent when U-937 cells were stimulated with LPS, probably due to accelerated contribution of the target signal-transduction cascade in the cytokine production. Together, these observations demonstrate the in vivo efficacy of JTE-607 on the cytokine production of AML cells, which may exacerbate the pathology of AML through the autocrine and paracrine signaling.

In conclusion, we demonstrated the unique antileukemic effect of JTE-607 in vivo using a sophisticated acute leukemia model. The results suggest JTE-607 exhibits its antileukemic effect by suppression of both proliferation and inflammatory cytokine production in AML cells. Although further experiments will be required to show the direct involvement of inflammatory cytokines produced from both AML blasts and host cells in the pathology of the leukemia model, the results in the present work revealed that JTE-607 is a promising antileukemic drug candidate that may bring about a new approach in the therapy of AML.

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Cord blood transplantation for acute myelogenous leukemia using a conditioning regimen consisting of granulocyte colony-stimulating factor-combined high-dose cytarabine, fludarabine, and total body irradiation

Tomonari A, Takahashi S, Ooi J, Nakaoka T, Takasugi K, Uchiyama M, Tsukada N, Konuma T, Iseki T, Tojo A, Asano S. Cord blood transplantation for acute myelogenous leukemia using a conditioning regimen consisting of granulocyte colony-stimulating factor-combined high-dose cytarabine, fludarabine, and total body irradiation.

Abstract: The cytotoxic effect of cytarabine (Ara-C) on myeloid leukemic cells is enhanced by concomitant use of granulocyte colony-stimulating factor (G-CSF) *in vitro*. The feasibility of a conditioning regimen consisting of G-CSF-combined 24 g/m^2 Ara-C, 90 mg/m^2 fludarabine, and 12 Gy total body irradiation was studied for five patients with acute myelogenous leukemia in cord blood transplantation (CBT). Graft vs. host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. After the conditioning regimen, 2.48×10^7/kg (2.28–3.53) of cord blood nucleated cells was infused. Neutrophil counts consistently $> 0.5 \times 10^9$/L was achieved 24 d (22–32) after CBT. Grade I stomatitis and gastrointestinal toxicities occurred in all patients. Grades I and II acute GVHD occurred in one and four patients, respectively, which resolved without steroid therapy. Sepsis and aspergillosis occurred in two and one patients, respectively. All patients were alive without leukemia relapse at a follow up of 15 months (12–43) after CBT. This conditioning regimen could avoid the toxicities of high-dose cyclophosphamide but might enhance the cytotoxic effect of Ara-C. Large-scale studies will be needed to determine the efficacy and safety of the conditioning regimen in CBT.

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Key words: granulocyte colony stimulating factor; cytarabine; acute myelogenous leukemia; cord blood, transplantation cardiac dysfunction

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The cytotoxic effect of cell-cycle-dependent agent cytarabine (Ara-C) on myeloid leukemic cells is enhanced by concomitant use of granulocyte colony stimulating factor (G-CSF) *in vitro* (1, 2). The clinical efficacy of a conditioning regimen consisting of G-CSF-combined 24 g/m^2 Ara-C and 12 Gy total body irradiation (TBI) for patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) was previously studied in bone marrow transplantation (BMT) from human leukocyte antigen (HLA)-matched sibling donors (1, 3, 4). The results suggested that the regimen was well feasible and might reduce post-transplant relapse in AML and MDS.

Recently, umbilical cord blood transplantation (CBT) from unrelated donors has been performed

increasingly for adult patients (5, 6). For patients with AML and MDS in CBT, we have used a conditioning regimen including G-CSF-combined 12 g/m^2 Ara-C, 120 mg/kg cyclophosphamide (CY), and 12 Gy TBI (7–9). Although this regimen was also shown to be well feasible, high-dose CY has possible adverse effects such as cardiotoxicity, in particular for patients with cardiac dysfunction before CBT (10, 11). In addition, this regimen contained only a half-dose of Ara-C when compared with the regimen for BMT.

To apply the efficacy of G-CSF-combined high-dose Ara-C for myeloid leukemic cells while avoiding toxic effects of high-dose CY, we studied the feasibility of an alternative conditioning regimen consisting of G-CSF-combined high-dose Ara-C, fludarabine, and TBI for patient with AML in CBT. In contrast to BMT from HLA-matched sibling donors, CBT from HLA-mismatched unrelated donors is associated with a high rate of graft failure (12). To enhance the immunosuppressive effect, the conditioning regimen in the present study included fludarabine at a dose of 90 mg/m^2 in addition to the previous regimen used for BMT from HLA-matched sibling donors.

Patients and methods

Patients

From March 2002 to October 2004, 24 patients with AML underwent CBT at our institution. Among them, 19 patients without cardiac dysfunction received a conditioning regimen containing high-dose CY. Because the remaining five patients had cardiac dysfunction at the time of transplantation (Table 1), they were avoided to receive high-dose CY as a conditioning regimen. The patients received a conditioning regimen consisting of G-CSF-combined high-dose Ara-C, fludarabine, and TBI. The patients did not have suitable HLA-matched related and unrelated donors for BMT, and thus were enrolled in our study. Written informed consent was obtained from each patient.

Transplantation procedures

Patients received 12 Gy TBI in four divided fractions on days −8 and −7, Ara-C (3 g/m^2 every 12 h for 4 d) with 5 μg/kg G-CSF (Lenograstim) from 12 h before the first dose of Ara-C to the end of Ara-C, as described previously (1, 3, 4). Administration of Ara-C was initiated at the night on day −6 or the morning on day −5. Fludarabine was administered intravenously at a dose of 30 mg/m^2 on days −5, −4, and −3. Graft vs. host disease (GVHD) prophylaxis consisted of cyclosporine (CSP) (3 mg/kg/d) with a short course of methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6). For bacterial and fungal infection prophylaxis, 450 mg/d tosufloxacin tosilate and 200 mg/d fluconazole or 100 mg/d itraconazole, respectively, were administered from day −14. For herpes simplex virus infection prophylaxis, 1000 mg/d acyclovir was administered orally from day −3 to +35. Cytomegalovirus (CMV) infection was monitored using a CMV antigenemia assay twice a week after engraftment (13). Based on the results of the CMV antigenemia assay, preemptive therapy with intravenous ganciclovir was initiated. To facilitate neutrophil engraftment, G-CSF (Lenograstim) was administered intravenously at a dose of 5 μg/kg/d from day +1 after CBT until sustained neutrophil engraftment was achieved.

Table 1. Characteristics of patients and CB grafts

	Patient number				
	1	2	3	4	5
Age (yr)	47	47	35	43	45
Gender	M	F	M	F	F
Body weight (kg)	76	59	61	45	45
Blood group	A	A	AB	AB	O
FAB classification	M2	M4	M2	M3	M4
Disease status	Rel3	CR1	CR2	CR2	CR2
LVEF (%)	44	53	53	45	45
Medication	ACEI	ACEI	ARB	ARB	ARB
CMV serostatus	P	P	P	P	P
CB graft					
TNC (×10^7/kg)	2.28	2.57	2.48	2.38	3.53
CD34 (×10^5/kg)	0.50	1.11	0.72	0.64	1.35
Gender	F	M	M	M	M
Blood group	A	O	A	A	O
HLA disparity	4	2	3	2	3

M, male; F, female; FAB, French–American–British; Rel3, third relapse; CR1, first complete remission; CR2, second complete remission; LVEF, left ventricular ejection fraction; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CMV, cytomegalovirus; P, positive; CB, cord blood; TNC, total nucleated cells.

Cord blood graft

The CB unit was chosen according to the number of nucleated cells per recipient's body weight and the HLA compatibility. The matching of HLA-A and B was confirmed by serologic typing methods. The matching of DRB1 was confirmed by genomic typing methods.

Study end points

The primary end points of the study were to evaluate toxicity and engraftment. The secondary end points were assessment of GVHD, day +100 transplantation-related mortality (TRM), relapse, and survival.

Post-transplant evaluation

Neutrophil engraftment was defined as an absolute neutrophil count (ANC) exceeding 0.5×10^9/L for three consecutive days. Platelet engraftment was defined as a platelet count exceeding 20×10^9/L for three consecutive days without platelet transfusion. The chimeric status after CBT was determined either by fluorescence *in situ* hybridization with a Y chromosome probe for sex-mismatched CBT or by polymerase chain reaction (PCR) analyses of polymorphic microsatellite regions for sex-matched CBT. Regimen-related toxicity (RRT) was graded using the Bearman scores (14). Both acute and chronic GVHD were graded according to previously published criteria (15, 16). Data were analyzed as of October 2005.

Results

Patients

Study patients were a median of 45 yr old (35–47) (Table 1). Two patients were males and three patients were females. One patient (no. 1) had AML in relapse and the remaining four patients had AML in complete remission (CR). They all had cardiac dysfunction with left ventricular ejection fraction (LVEF) of $<55\%$ as determined by echocardiograph before CBT. Two patients received angiotensin converting enzyme inhibitor (ACEI) therapy and three patients received angiotensin receptor blocker therapy for cardiac dysfunction before CBT.

Engraftment

The median doses of total nucleated cells and CD34-positive cells before freezing were 2.48×10^7/kg (2.28–3.53) and 0.72×10^5/kg (0.50–1.35), respectively (Table 1). An ANC exceeding 0.5×10^9/L was achieved a median of 24 d (22–32) after CBT (Table 2). A platelet count exceeding 20×10^9/L was achieved a median of 31 d (30–75) after CBT. All patients achieved full donor chimerism on the first bone marrow examination after CBT on a median of 41 d (26–47) after CBT. The delayed neutrophil and platelet engraftment in patient no. 2 was probably due to CMV infection and subsequent antiviral treatment before engraftment.

Toxicity

All patients developed grade I stomatitis and gastrointestinal toxicities (Table 2). However, no patients developed grade I or more cardiac, bladder, renal, pulmonary, hepatic, or central nervous system toxicities which were attributable to the conditioning regimen. One patient (no. 4) developed symptomatic congestive heart failure on day +137, which resolved by the administration of diuretics and ACEI, and the discontinuation of CSP. Although the etiology was uncertain, the conditioning regimen seemed not to be associated with the development of this late-onset heart failure. The remaining four patients did not develop symptomatic congestive heart failure after CBT.

Table 2. Results

	Patient number				
	1	2	3	4	5
Engraftment (d)					
Neutrophil	22	32	24	23	25
Platelet	28	75	30	31	39
aGVHD (grade)	I	II	II	II	II
Skin (stage)	2	3	3	3	3
Gut (stage)	0	0	1	0	0
Liver (stage)	0	0	0	0	0
cGVHD	L	L	L	L	No
Toxicity (grade)					
Cardiac	No	No	No	No	No
Bladder	No	No	No	No	No
Renal	No	No	No	No	No
Pulmonary	No	No	No	No	No
Hepatic	No	No	No	No	No
CNS	No	No	No	No	No
Stomatitis	I	I	I	I	I
Gastrointestinal	I	I	I	I	I
Infection (onset day)					
Sepsis	7	7	No	No	No
Aspergillosis	No	No	No	No	18
CMV	31	35	46	36	42
VZV	No	No	No	120	No
HSV	No	144	106	120	No
Survival (months)	43	26	15	12	12
Status	Alive	Alive	Alive	Alive	Alive

aGVHD, acute graft-vs.-host disease; cGVHD, chronic graft-vs.-host disease; L, limited-type; CNS, central nervous system; CMV, cytomegalovirus; VZV, varicella-zoster virus; HSV, herpes simplex virus.

Graft vs. host disease

One patient (no. 1) developed grade I acute GVHD and the remaining four patients developed grade II acute GVHD. However, all patients did not need steroid therapy. Four patients (nos 1–4) developed limited-type chronic GVHD.

Infection

Two patients (nos 1 and 2) developed sepsis by *Enterococcus fecalis* and *fecium*, respectively, both on day +7. Both patients were successfully treated with antibiotics. One patient (no. 5) developed invasive pulmonary aspergillosis on day +18. She was successfully treated with a combination of itraconazole, micafangin, and liposomal ampho-

tericin B. In all patients, CMV infection was documented by an antigenemia assay at a median of 36 d (31–46) after CBT. In one patient (no. 1), CMV antigenemia resolved spontaneously without ganciclovir therapy. In the remaining patients (nos 2–5), CMV antigenemia was successfully treated with pre-emptive therapy with ganciclovir. No patients developed CMV disease. One patient developed localized cutaneous varicella-zoster infection on day +120, and was successfully treated with acyclovir therapy. Two patients (nos 2 and 4) developed oral herpes simplex virus infection on days +144 and +106, respectively, and were successfully treated with acyclovir therapy.

Survival

All patients were alive without leukemia relapse at a median follow up of 15 months (12–43) after CBT.

Discussion

In the present study, we reported five patients with AML who underwent CBT following a conditioning regimen consisting of G-CSF-combined 24 g/m^2 Ara-C, 90 mg/m^2 fludarabine, and 12 Gy TBI. Engraftment and full donor chimerism after CBT were achieved in all patients. The median day of neutrophil engraftment was 24 d after CBT, which seemed to be comparable with 22 and 23 d in our previous studies (7–9). RRT within 100 d after CBT and acute GVHD were well tolerated. All patients were alive without disease progression. The disease status in four of five patients (nos 2–5) was the first or second CR, which would also contribute to the high rate of disease-free survival after CBT. In addition, the graft-vs.-leukemia effect might play a role for preventing leukemia relapse after CBT (5, 6, 9). Although the patient number was too small, these results suggested that this conditioning regimen was feasible. This conditioning regimen could avoid the toxicities of high-dose CY even for patients with cardiac dysfunction but might enhance the cytotoxic effect of Ara-C.

The clinical efficacy of G-CSF-combined high-dose Ara-C for patients with AML and MDS was previously studied by using two conditioning regimens. First, a conditioning regimen consisting of G-CSF-combined 24 g/m^2 Ara-C and 12 Gy TBI was applied to patients with AML and MDS in BMT from HLA-matched sibling donors (1, 3, 4). Next, a conditioning regimen consisting of G-CSF-combined 12 g/m^2 Ara-C, 120 mg/kg CY, and 12 Gy TBI was applied to patients with AML and MDS in CBT from HLA-mismatched unrelated donors (7, 8). These studies showed that the relatively high rates of survival and low rates of relapse and TRM, suggesting that the conditioning regimens with G-CSF-combined high-dose Ara-C were well feasible and might reduce post-transplant relapse in patients with AML and MDS.

High-dose CY used in a conditioning regimen for hematopoietic stem cell transplantation (SCT) can induce significant cardiac toxicity in a dose-dependent manner (10). Thus, we first chose patients with cardiac dysfunction in the present study. In all five patients, LVEF before CBT was reduced to < 55%. Fujimaki et al. (11) showed that patients with a reduced LVEF of 55% or less before SCT were at significant risk of severe cardiac toxicity after a CY-containing conditioning regimen. However, it is controversial whether a reduced LVEF before SCT increases the risk of CY-induced cardiac toxicities (17, 18). In our institution, the study on the association between various conditioning regimens and changes of cardiac function after SCT is under way.

One of the major disadvantages in CBT is a high rate of graft failure, particularly in patients with chronic myelogenous leukemia (CML) or severe aplastic anemia (12). Although our results were obtained from only five patients with AML, the conditioning regimen provided sustained engraftment with full donor chimerism and acceptable toxicities. Large-scale studies with a prolonged follow up will be needed to determine the efficacy and safety of this alternative conditioning regimen for CBT.

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