resistance of T-cells in IL-2 depeletion[3]. Then, activated T cells were subjected to an apoptosis assay by FAS stimulation or IL-2 depletion. Remarkable resistance to IL-2 depletion but not to FAS-dependent apoptosis (Fig. 1d and e) was seen. This was in contrast to T cells from FAS mutated ALPS type 1a which showed remarkable resistance to FAS dependent apoptosis and normal apoptosis induction by IL-2 withdrawal (Fig. 1d and e). Western blotting analysis of activated T cells or Epstein-Barr virus-transformed B cells showed reduced expression of Bim (Fig. 1f).

In case 2, autoimmune phenotype and hepatosplenomegaly were remarkable as shown in Supplementary data 1. The patient was initially diagnosed as Evans Syndrome based on presence of hemolytic anemia and autoimmune thrombocytopenia. DNT cells were 1.1% of TCRαβ cells in the peripheral blood, which did not meet with the criteria of ALPS. Although spontaneous colony formation was shown in PB- and BM-MNC, and GM-CSF hypersensitivity was demonstrated in BM-MNC derived CD34 positive cell (Supplemental data 1 Table2), she showed no massive monocytosis or increased HbF. Thus the diagnosis was less likely to be ALPS or JMML. DNA sequencing of JMML related genes such as *NRAS, KRAS, HRAS, PTPN11*, and *CBL* identified somatic but not germline KRAS G13D mutation (Fig. 1b). KRAS G13D mutation was detected in granulocytes and T cells. Mutation was not identified in B cells by conventional DNA sequencing (data not shown). Mutant allele specific quantitative PCR revealed mutated allele was almost equally present in granulocytes and T cells, but barely in B cells (Fig. 1c). Activated T cells showed resistance to IL-2 depletion but not to FAS-dependent apoptosis (Fig. 1d and e). Both of our cases were characterized by strong autoimmunity, immune cytopenia and lymphadenopathy or hepatosplenomegaly with partial similarity with ALPS or JMML. However, they did not meet with the well defied diagnostic

criteria of ALPS[2] or JMML[6]. It is interesting that Case 2 presented GM-CSF hypersensitivity, which is one of the hallmarks of JMML. Given the strict clinical and laboratory criteria of JMML and ALPS, our two cases should be defined as a new disease entity, like RAS associated ALPS like disease (RALD). Recently defined NRAS mutated ALPS type IV may also be included in a similar disease entity.

There are several cases of JMML reported simultaneously having clinical and laboratory findings compatible with autoimmune disease[8,9]. Autoimmune syndromes are occasionally seen in patients with myelodysplastic syndromes, including chronic myelomonocytic leukemia[10]. These previous findings may suggest a close relationship of autoimmune disease and JMML. Since KRAS G13D has been identified in JMML[11-13], it is tempting to speculate that KRAS G13D mutation is involved in JMML as well as RAS associated ALPS like disease (RALD). It should be noted in JMML, erythroid cells reportedly carry mutant RAS, while B and T cell involvement was variable[13]. In both of our cases, myeloid cells and T cells carried mutant RAS, while B cells were affected variably. These findings would support a hypothesis that the clinical and hematological features are related to the differentiation stages of hematopoietic stem cells where RAS mutation is acquired. JMML-like myelo-monocytic proliferation may predict an involvement of RAS mutation in myeloid stem/precursor cell level whereas ALPS-like phenotype may predict that of stem/precursor cells of lymphoid lineage, especially of T cells. Under the light of subtle differences between the two cases presented, their hematological and clinical features may reflect the characteristics of the stem cell level where *KRAS* mutation is acquired. Involvement of the precursors with higher propensity toward lymphoid lineage may lead to autoimmune phenotypes, while involvement of those with propensity toward the myeloid lineage may lead to GM-CSF hypersensitivity while still

sharing some overlapping autoimmune characteristics.

One may argue from the other points of view with regard to the clinicopathological features of these disorders. First, transformation in a fetal HSC might be obligatory for the development of JMML[14] and that in HSC later in life may not have the same consequences. Second, certain KRAS mutations may be more potent than the others. Codon 13 mutations are generally less deleterious biochemically than codon 12 substitutions, and patients with JMML with codon 13 mutations have been reported to show spontaneous hematologic improvement[12,15]. Thus further studies are needed to reveal in-depth clinicopathological characteristics in this type of lympho-myelo proliferative disorders.

KRAS mutation may initiate the oncogenic pathway as one of the first genetic hits, but is insufficient to cause frank malignancy by itself[16,17]. Considering recent findings that additional mutations of the genes involved in DNA repair, cell cycle arrest, and apoptosis are required for full malignant transformation, one can argue that RAS associated ALPS like disease (RALD) patients will also develop malignancies during the course of the diseases. Occasional association of myeloid blast crisis in JMML and that of lymphoid malignancies in ALPS will support this notion. Thus the two patients are now being followed up carefully. It was recently revealed that half of the patients diagnosed with Evans syndrome, an autoimmune disease presenting with hemolytic anemia and thrombocytopenia, meet the criteria for ALPS diagnosis[18,19]. In this study, FAS-mediated apoptosis analysis was utilized for the screening. Considering the cases we presented, it will be intriguing to re-evaluate Evans syndrome by IL-2 depletion-dependent apoptosis assay focusing on the overlapping autoimmunity with RAS associated ALPS like disease (RALD).

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Author ship

MT and SM designed entire experiments and wrote this manuscript. KS, NM, and MT treat those patients, and designed clinical laboratory test. JP performed experiments described in Fig.1b-f. KM, HM, and SD performed colony and mutational analysis. MN, TM, KK, SK, YK and AT supervised clinical and immunological experiments, or coordinated clinical information.

Conflict of interest disclosure

The authors declare no conflict of interest.

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Figure Legends

Figure 1

a. Flow cytometric analysis of DNT cells. CD8 and CD4 double staining was performed in TCRαβ-expressing cells.

b. Electropherogram showing KRAS G13D mutation in BM-MNC in case 1 (left panel) and case 2 (right panel).

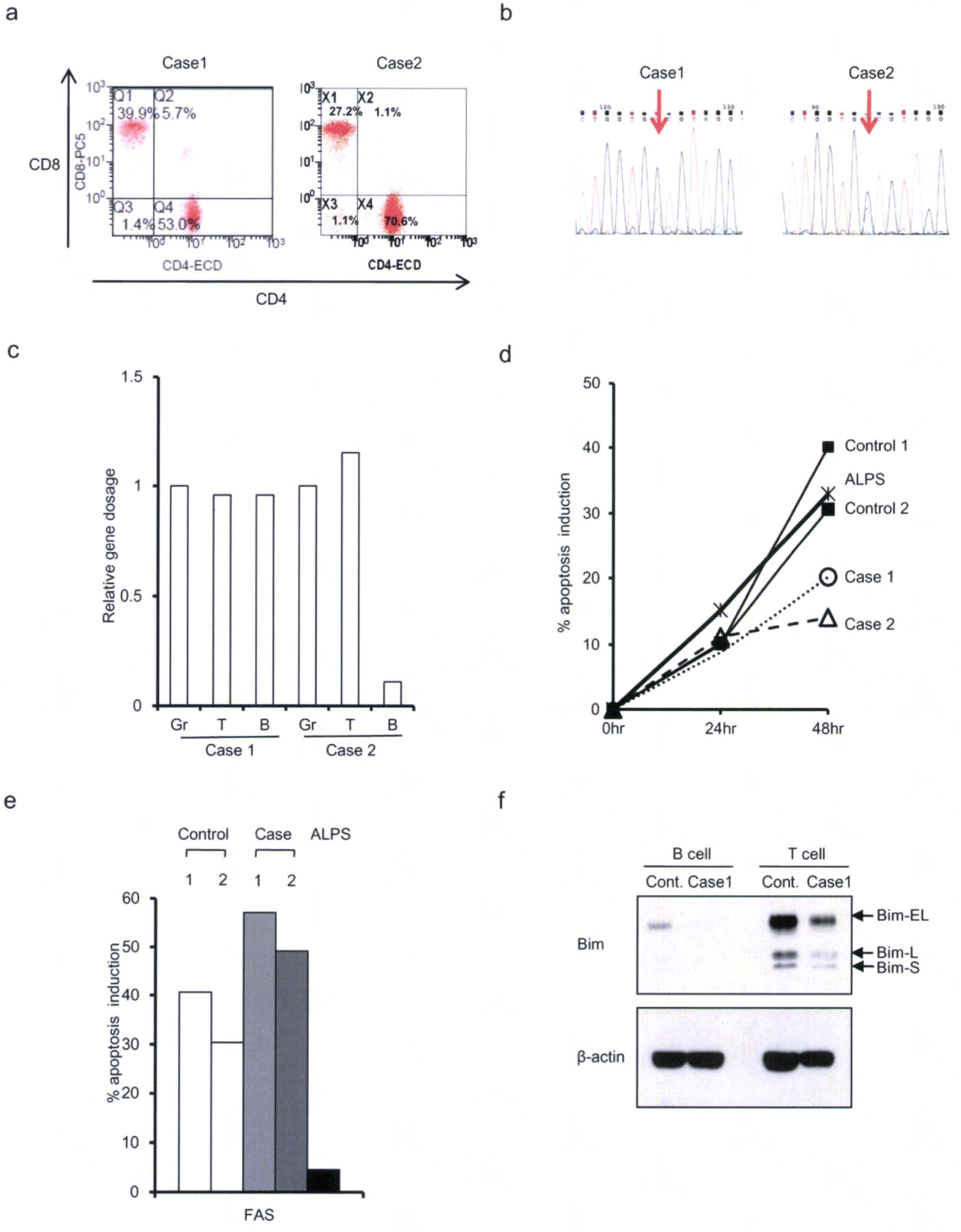
c. Gene dosage of mutated allele in granulocyte (Gr), T cell (T) and B cell (B). Relative gene dosage was estimated by a mutant allele specific PCR method in case 1 and 2 using albumin gene as internal control.

d. Apoptosis assay using activated T cells. Apoptosis percent was measured by flow cytometry with Annexin V staining 24 and 48 hr after IL-2 depletion

e. Apoptosis percent was measured 24hr after addition of anti-FAS CH11 antibody (final 100ng/ml)

f. Western blotting analysis of Bim expression.

Figure 1


a
Case1
Case2
CD8
CD8-PC5
Q1
39.9%
Q2
5.7%
Q3
1.4%
Q4
53.0%
X1
27.2%
X2
1.1%
X3
1.1%
X4
70.6%
CD4-ECD
CD4
b
Case1
Case2
c
Relative gene dosage
1.5
1
0.5
0
Gr
T
B
Gr
T
B
Case 1
Case 2
d
% apoptosis induction
50
40
30
20
10
0
0hr
24hr
48hr
Control 1
ALPS
Control 2
Case 1
Case 2
e
Control
Case
ALPS
1
2
1
2
% apoptosis induction
60
50
40
30
20
10
0
FAS
f
B cell
T cell
Cont.
Case1
Cont.
Case1
Bim
Bim-EL
Bim-L
Bim-S
β-actin

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

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

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

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

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

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

introduction

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

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

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

patients and methods

patient and donor eligibility

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

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

study design and dose determination

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

study endpoints and definitions

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

conditioning regimens

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

GVHD prophylaxis and grading

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

chimerism analysis

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

statistical analysis

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

results

patient and donor characteristics

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

Table 1. Patient and donor characteristics

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

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

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

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

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

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

analysis of primary endpoint: conditioning regimen, engraftment and chimerism

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

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

secondary endpoints

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

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

Table 2. Mel dose, engraftment and chimerism

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

Patient 2 was not listed because of protocol violation.
Mel, melphalan; HLA, human leukocyte antigen; Y, yes; N, no; m, months; d, days; NHL, non-Hodgkin's lymphoma; N.A., not available; D, dead; CNS, central nervous system; 1mis, 1 locus mismatch; A, alive; N.E., not evaluable; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia.

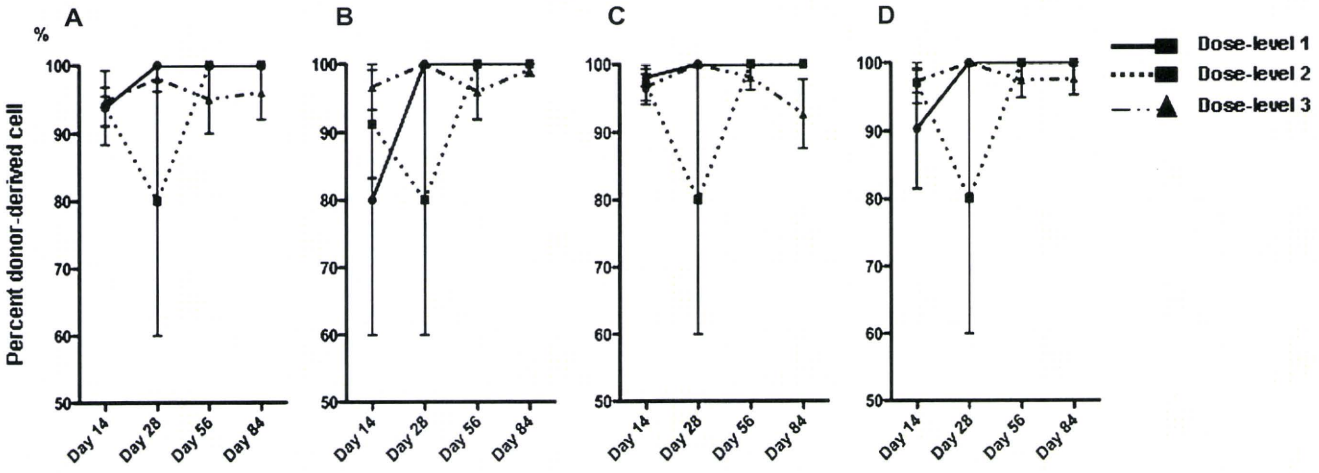

Figure 1. Serial measurement of lineage-specific peripheral blood chimerism. (A) CD3+ T-cell fraction. (B) CD33+ polymorphonuclear cell fraction. (C) CD16+/CD56+ NK-cell fraction. (D) Flowthrough fraction. The mean (±SEM) percentage of donor-derived cells is plotted for each dose-level group. The solid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

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

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

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

Table 3. Toxicity by melphalan dose level

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

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

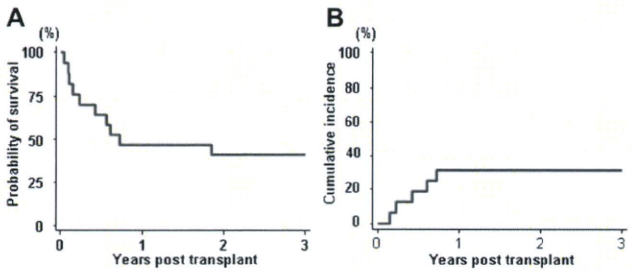

Figure 2. (A) Kaplan-Meier estimate of EFS of all assessable patients. Probability of EFS was 50% (95% CI 25% to 71%) at 1 year and 44% (95% CI 20% to 67%) at 3 years after transplant. (B) Cumulative incidence of relapse was 31% (95% CI 11% to 54%) at 1 year after transplant. EFS, event-free survival;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

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

discussion

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

Table 4. Causes of death

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

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

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

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

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

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

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

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

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disclosure

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Cytotoxic T-lymphocyte antigen 4 haplotype correlates with relapse and survival after allogeneic hematopoietic SCT

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CTLA-4 is a negative regulator of activated T cells and the association of CTLA-4 polymorphisms with autoimmune diseases and transplant outcome has been reported. We evaluated the effect of donor CTLA-4 polymorphisms on outcome after allogeneic hematopoietic SCT (HSCT). We analyzed 147 Japanese HLA-matched sibling recipients and their donors who had undergone allogeneic HSCT. Genotyping of three single-nucleotide polymorphisms in CTLA-4 (318, 49, CT60) was performed using TaqMan-PCR. According to the international HapMap database, only these three CTLA-4 haplotypes, classified as C-G-G, C-A-A and T-A-G, are present in the Japanese population. In this study, percentage expression of the C-G-G, C-A-A and T-A-G haplotypes was 59.5, 30.6 and 9.9%, respectively. Recipients of the C-A-A haplotype donor showed a significantly lower risk of relapse (HR: 0.54, 95% CI: 0.30–0.97, P 0.040) and a trend toward higher OS (HR: 0.61, 95% CI: 0.36–1.0, P 0.054) than did recipients of a donor without the C-A-A haplotype. The presence or absence of the C-A-A haplotype did not affect GVHD or non-relapse mortality. As the presence of the C-A-A haplotype reduced relapse risk and improved survival after allogeneic HSCT, this CTLA-4 haplotype may provide useful information for donor selection.
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Introduction

Allogeneic hematopoietic SCT (HSCT) has been established as an effective treatment for patients with hematological malignancies. GVHD caused by donor-derived T cells is one of the most common causes of morbidity and mortality after allogeneic HSCT.[1] However, donor-derived T cells also mediate a GVL effect, which assists in the eradication of tumor cells.[2] Control of alloimmune reactions and separation of the potent GVL effect from severe GVHD are therefore important for a successful outcome after allogeneic HSCT. Although optimal HLA matching between patients and donors is critical for the prevention of severe GVHD, this can still develop after HSCT from an HLA-identical sibling donor due to non-HLA gene polymorphisms.[3,4] Thus, the association of polymorphisms in genes encoding mHA,[5,6] cytokines,[7,8] chemokines[9] and drug-metabolizing enzymes[10] with transplant outcomes has been reported.

CTLA-4 is a receptor expressed on the surface of activated T cells, and is a homolog of CD28 that is responsible for T-cell activation. Although both CTLA-4 and CD28 bind the two ligands B7.1 (CD80) and B7.2 (CD86) expressed on APCs, CTLA-4 binds B7 molecules with higher affinity and avidity than CD28. CTLA-4 gene polymorphisms correlate with autoimmune diseases such as systemic lupus erythematosus,[11–13] type 1 diabetes mellitus[14,15] and Graves' disease.[16] In addition, recent studies have shown an association of the CTLA-4 polymorphisms (318 (rs5742909), 49 (rs231775) and CT60 (rs3087243)) with outcome after allogeneic HSCT.[17–19] We therefore focused our study on these three polymorphisms, and analyzed the impact of donor genotypes and haplotypes in the Japanese population on outcome after HLA-identical sibling HSCT.

Patients and methods

Patients

The study population included adult Japanese patients who received hematopoietic stem cells from an HLA-identical sibling donor for the treatment of hematological

Table 1 Patient characteristics

No. of patients	147
Median age in years (range)	39 (15–62)
Sex (M/F)	81/66
Disease	
AML	49
ALL	28
CML	39
Myelodysplastic syndrome	17
Malignant lymphoma	6
Multiple myeloma	5
Others	3
Disease risk	
Standard[a]	81 (55%)
High[b]	66 (45%)
Graft source	
BM	110 (75%)
Peripheral blood	37 (25%)
Preconditioning	
Myeloablative	128 (87%)
Reduced-intensity	19 (13%)
Acute GVHD	
0	82 (56%)
I	37 (25%)
II	19 (13%)
III-IV	9 (6%)
Chronic GVHD	
None	74 (53%)
Limited	11 (8%)
Extensive	54 (39%)
Relapse at 5 years	40%
Non-relapse mortality at 5 years	21%
OS at 5 years	50%

[a]Acute leukemia in first CR, CML in first chronic phase and myeloid displastic syndrome with IPSS score of 1.0 or lower.
[b]More advanced status than standard-risk disease.

malignancies at the Nagoya University Hospital and the Japanese Red Cross Nagoya First Hospital between 1987 and 2006. A total of 147 recipient-donor pairs were selected according to the following criteria: (1) DNA samples and clinical data were available; (2) an unmanipulated graft was transplanted; and (3) short-term MTX and CsA were used as GVHD prophylaxis. MTX was administered i.v. on day 1 (10 mg/m^2) and on days 3 and 6 (7 mg/m^2 each day). CsA was administered by i.v. infusion at a dose of 3.0 mg per kg at beginning on day 1.

Patient characteristics are summarized in Table 1. A total of 81 patients (55%) were classified as having standard-risk disease defined as acute leukemia in first CR, CML in first chronic phase and myelodysplastic syndrome with an international prognostic scoring system (IPSS) score of 1.0 or lower, whereas 66 patients (45%) had high-risk disease defined as disease of more advanced status than standard risk disease. Graft source was BM for 110 patients (75%) and peripheral blood for 37 patients (25%). Conditioning was myeloablative for 128 patients (87%) and reduced-intensity for 19 patients (13%).

Informed consent was obtained from all patients and donors. The study was approved by the ethics committees at the Nagoya University Hospital, the Japanese Red Cross Nagoya First Hospital and the Tokai University School of Medicine.

CTLA-4 genotyping

Genomic DNA was obtained from donor peripheral blood or BM using the QIAamp DNA Blood Mini Kit (QIAGEN sciences, Germantown, MD, USA). The TaqMan PCR method was used to determine the three single-nucleotide polymorphism (SNP) genotypes of CTLA-4: 318 (rs5742909), 49 (rs231775) and CT60 (rs3087243). The respective primers and probes used for the TaqMan PCR were: 318 C/T, forward 5'-AAATGAATTGGACTGG ATGGT-3' and reverse 5'-TTACGAGAAAGGAAGCC GTG-3', probe 5'-GTCTCCACTTAGTTATCCAGATCC T[C/T]AAAGTGACATGAAGCTTCAGTTTC-3'; 49 A/G, forward 5'-GCTCTACTTCCTGAAGACCT-3' and reverse 5'-AGTCTCACTCACCTTTGCAG-3', probe 5'-G CACAAGGCTCAGCTGAACCTGGCT[A/G]CCAGGA CCTGGCCCTGCACTCTCCT-3'; CT60 A/G, forward 5'-ATCTGTGGTGGTCGTTTTCC-3' and reverse 5'-CC ATGACAACTGTAATGCCTGT-3', probe 5'-TCTTCAC CACTATTTGGGATATAAC[A/G]TGGGTTAACACAG ACATAGCAGTCC3'.

PCR reactions were performed in a 10-mL reaction volume containing 1 TaqMan Universal Master Mix (Applied Biosystems, Tokyo, Japan), 1 mmol of each primer, 1 mL of each probe and 1 mL of genomic DNA. Thermal cycle conditions were 95 1C for 10 min, 40 cycles of 92 1C for 15 s and 60 1C for 1 min. All PCR and endpoint fluorescent readings were analyzed using an ABI7900 sequence detection system (Applied Biosystems).

Statistical analysis

OS was calculated from the date of transplantation to the date of death using the Kaplan–Meier method, and P-values were calculated using a log-rank test. EFS was calculated from the date of transplantation to the date of death or relapse, whichever occurred first, and P-values were calculated using a log-rank test. Non-relapse mortality (NRM) was defined as mortality due to any cause other than relapse or disease progression. Cumulative incidences of NRM and relapse were estimated using Gray's test, with relapse and NRM, respectively, as a competing risk.

Acute GVHD was diagnosed and graded according to consensus criteria.[20] Chronic GVHD was evaluated in patients who survived beyond day 100, and was categorized as limited or extensive.[21] A multivariate Cox model was created for analysis of grade II-IV acute GVHD, grade III-IV acute GVHD, chronic GVHD, OS, NRM, relapse and EFS using stepwise selection at a significance level of 5%. Age, sex, disease risk, conditioning regimen and graft source were used as covariates, and those variables with a P-value of less than 0.2 in the univariate analysis were entered into the stepwise selection method. Hazard ratios of the CTLA-4 haplotype CAA were adjusted using these models. Analysis was carried out using STATA (StataCorp. 2007; Stata Statistical Software: Release 10.0. Special Edition. Stata Corporation, College Station, TX, USA). P-values of less than 0.05 were

regarded as statistically significant, and P-values between 0.05 and 0.1 as suggesting a trend.

Results

Frequencies of CTLA-4 genotypes and haplotypes

Frequencies at which the three CTLA-4 SNPs were expressed in the 147 donors are listed in Table 2. The SNPs 318 (rs5742909), 49 (rs231775) and CT60 (rs3087243) were included in one haplotype block that was constructed using the international HapMap database. The haplotype analysis revealed only three haplotypes in the Japanese population: 318*C/ 49*G/CT60*G (C-G-G), 318*C/ 49*A/CT60*A (C-A-A) and 318*T/ 49*A/CT60*G (T-A-G). In this cohort, the frequencies of the haplotype C-G-G, C-A-A and T-A-G were 59.5, 30.6 and 9.9%, respectively. All of the donors were distributed among the six haplotype combinations (Table 3).

Effect of the CTLA-4 haplotype C-A-A on transplant outcome

It has been shown that the donor 318 C allele is associated with a lower risk of relapse[19] and that the donor CT60 AA genotype is associated with a lower risk of relapse and a higher OS.[17] We therefore focused our analysis on the C-A-A haplotype, and examined the association between the C-A-A haplotype and the outcome after allogeneic HSCT.

The incidence of grade II-IV acute GVHD was 19% for all patients (Table 1). There was no significant difference between the cumulative incidences of grade II-IV acute GVHD in patients who received stem cells from a donor with the C-A-A haplotype (21%) or from a donor without the C-A-A haplotype (17%) (P 0.66) (Figure 1a).

Of 147 patients, 139 could be evaluated for chronic GVHD, and the incidence of chronic GVHD was 47% (Table 1). The incidence of chronic GVHD was not significantly different in the presence or absence of the C-A-A haplotype (51 vs 47%, P 0.81) (Figure 1b). Recipients of donors with the C-A-A haplotype showed a significantly lower incidence of relapse (28 vs 45%, P 0.049) and a higher OS (58 vs 36%, P 0.033) than recipients of donors without the C-A-A haplotype (Figures 2a and b). However, there was no significant difference in NRM between recipients of donors with or without the C-A-A haplotype (17% for both) (P 0.87).

Multivariate analyses showed that age 40 years was a risk factor for chronic GVHD, NRM, OS and EFS; that high-risk disease was a risk factor for relapse, OS and EFS; and that reduced intensity conditioning was a risk factor for chronic GVHD and relapse; and that PBSCT was a risk factor for acute and chronic GVHD. The hazard ratios of the C-A-A haplotype, adjusted by these factors, are listed in Table 4. The C-A-A haplotype was significantly associated with a lower relapse rate (HR: 0.54, 95%, CI: 0.30–0.97, P 0.040). Additionally, the group with the C-A-A haplotype exhibited trends toward higher OS (HR: 0.61, 95%, CI: 0.36–1.0, P 0.054) and EFS (HR: 0.67, 95%CI: 0.41–1.1, P 0.1), compared with the group without the C-A-A haplotype. The presence or absence of the C-A-A haplotype did not affect the incidence of acute or chronic GVHD or NRM.

Table 2 Frequency of CTLA-4 genotypes

Polymorphism	n (%)
No. of donors	147
318	
CC	121 (82.4)
CT	23 (15.6)
TT	3 (2.0)
+49	
GG	51 (34.7)
AG	73 (49.7)
AA	23 (15.6)
CT60	
GG	69 (46.9)
AG	66 (44.9)
AA	12 (8.2)

Table 3 Frequencies of the CTLA-4 haplotype

Haplotype	318	+49	CT60	n (%)
C-G-G/C-G-G	CC	GG	GG	51 (34.7)
C-G-G/C-A-A	CC	AG	AG	58 (39.5)
C-G-G/T-A-G	CT	AG	GG	15 (10.2)
C-A-A/C-A-A	CC	AA	AA	12 (8.2)
C-A-A/T-A-G	CT	AA	AG	8 (5.4)
T-A-G/T-A-G	TT	AA	GG	3 (2.0)

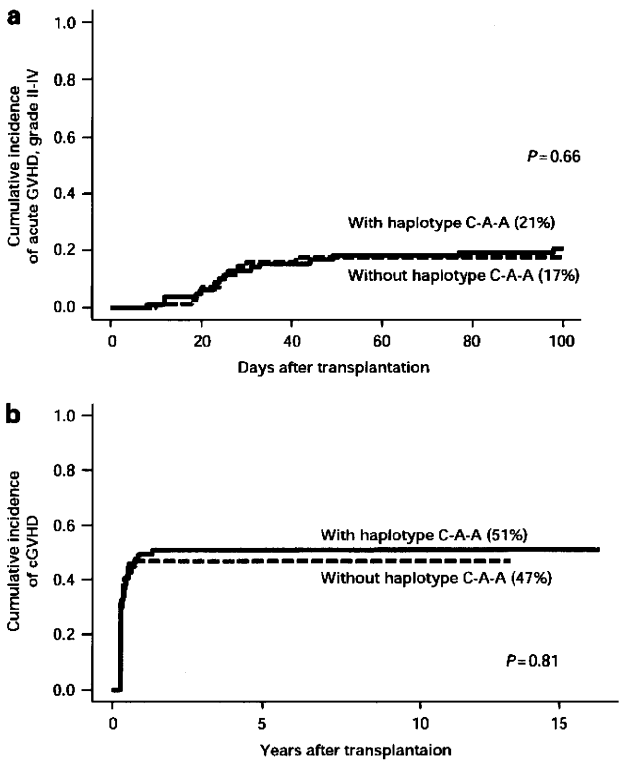

Figure 1 Association of (a) the cumulative incidence of grade II-IV acute GVHD and (b) chronic GVHD in recipients of donors with (solid line) and without (dotted line) the CTLA-4 C-A-A haplotype.

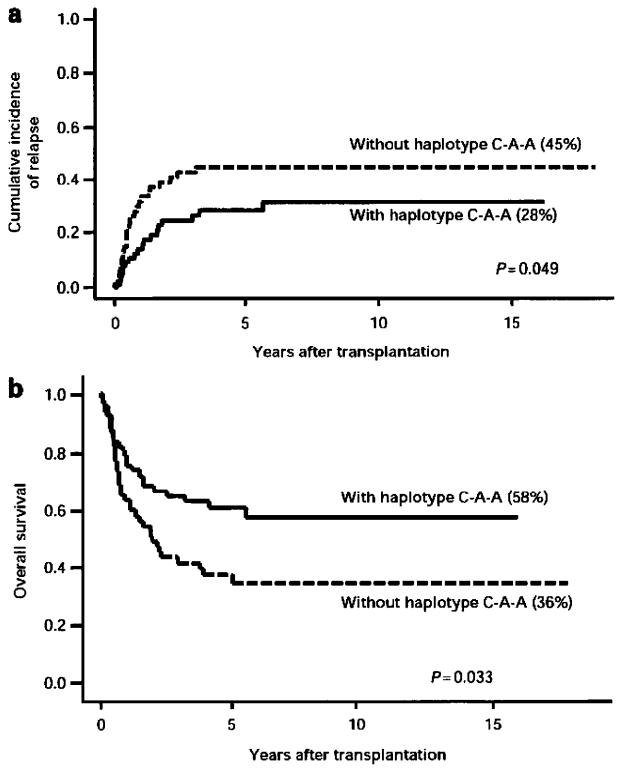

Figure 2 The impact of donors with (solid line) or without (dotted line) the CTLA-4 haplotype C-A-A on the incidence of relapse (a) and the OS (b) in recipient.

Table 4 Effect of donor CTLA-4 haplotype on transplant outcome

Events	Factors	Multivariate[a]	
		Hazard ratio (CI)	P-value
Acute GVHD Grade II-IV	PBSCT	3.4 (1.6–7.1)	0.001
	Haplotype C-A-A[b]	1.1 (0.53–2.4)	0.77
Acute GVHD Grade III-IV	PBSCT	6.1 (1.5–24)	0.011
	Haplotype C-A-A[b]	1.5 (0.38–6.0)	0.56
Chronic GVHD	Age≤40	1.7 (1.0–2.8)	0.05
	PBSCT	2.0 (1.1–3.5)	0.027
	RIC	0.28 (0.11–0.70)	0.0067
	Haplotype C-A-A[b]	1.0 (0.63–1.7)	0.92
Non-relapse mortality	Age≤40	2.4 (1.0–5.8)	0.042
	Haplotype C-A-A[b]	0.90 (0.39–2.0)	0.79
Relapse	High risk	2.6 (1.3–4.9)	0.005
	RIC	2.3 (1.2–4.5)	0.014
	Haplotype C-A-A[b]	0.54 (0.30–0.97)	0.04
OS	High risk	1.8 (1.1–3.1)	0.025
	Age≤40	1.9 (1.1–3.3)	0.013
	Haplotype C-A-A[b]	0.61 (0.36–1.00)	0.054
EFS	High risk	2.0 (1.3–3.4)	0.004
	Age≤40	1.6 (1.0–2.7)	0.046
	Haplotype C-A-A[b]	0.67 (0.41–1.1)	0.1

Abbreviation: RIC reduced intensity conditioning.
[a]Age, sex, disease risk, conditioning regimen, graft source were used as covariates.
[b]Adjusted by significant factors.

Discussion

Our results highlight the impact of the donor CTLA-4 haplotype 318*C/ 49*A/CT60*A (C-A-A) on outcome after allogeneic HSCT from an HLA-identical sibling.

It has been shown that the donor 318 C allele is associated with a lower risk of relapse[19] and that the donor CT60 AA genotype correlates with a lower risk of relapse and a higher OS.[17] Therefore, we focused on the C-A-A haplotype from three different haplotypes in the Japanese population, and examined the association between the C-A-A haplotype and the outcome after allogeneic HSCT. The presence of the CTLA-4 C-A-A haplotype exhibited a significantly lower incidence of relapse and a trend toward of a higher survival rate compared with the absence of the haplotype C-A-A, suggesting that the C-A-A haplotype might suppress the inhibitory function of CTLA-4 on tumor-reactive T cells and enhance the GVL effect.

The mechanism by which the C-A-A haplotype exerts its positive effects is still unclear. Several studies addressing the functional consequences of CTLA-4 SNP-318 and CT60 have been reported. The SNP 318 is located at the CTLA-4 promoter region, and the association of these alleles with promoter activity has been examined. Previous studies showed that the 318 C allele correlates with a lower promoter activity and a lower CTLA-4 expression than those observed with the 318 T allele.[22,23] The CTLA-4 gene is composed of four exons and has two isoforms: a full-length isoform (flCTLA-4) and a soluble form (sCTLA-4) that lacks exon 3, which encodes the transmembrane domain. Serum levels of sCTLA-4 increase in patients with various autoimmune diseases[24,25] and sCTLA-4 has the potential to bind to CD80/CD86,[26,27] suggesting that sCTLA-4 blocks the interaction of flCTLA-4 with CD80/CD86 and thereby enhances T-cell activation. It has been reported that the CT60 A allele is associated with a higher level of the sCTLA-4 mRNA than the CT60 G allele.[17,28] These results indicate that the 318 C allele and the CT60 A allele contribute to the reduction in CTLA-4 inhibitory function and to T-cell activation. However, association of the 49 A allele with a higher expression of CTLA-4 and augmentation of CTLA-4 inhibitory function has been reported.[16,29] Therefore, further investigation is required to elucidate the effect of the C-A-A haplotype on the anti-tumor activity of donor-derived T cells.

Although the C-A-A haplotype was associated with a low incidence of relapse, in this study it did not affect the incidence of GVHD, suggesting that the C-A-A haplotype may have the potential to separate GVL from GVHD responses. However, it might be because of our small cohort, as Perez-Garcia et al.[17] demonstrated that the donor CT60 AA genotype was associated with an increased risk of grade II-IV acute GVHD in a large cohort of 536 donors. All of the patients in our study were Japanese, and many of them (75%) had received BM as a stem-cell graft. It is known that Japanese patients have a lower risk of developing acute GVHD,[30] and that BMT is associated with a decrease in the development of acute GVHD.[31]

Thus, the ethnic population or the stem-cell source might also affect the association between CTLA-4 polymorphisms and the development of acute GVHD.

In summary, the presence of the CTLA-4 C-A-A haplotype reduced the risk of relapse and improved survival after allogeneic HSCT. Therefore, knowledge of the CTLA-4 haplotype may provide useful information for donor selection. The exact effect of the CTLA-4 C-A-A haplotype on transplant outcome should be determined in different cohorts with a substantially larger number of subjects.

Conflict of interest

The authors declare no conflict of interest.

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