Table 2 HSCT type and causes of death

Conditioning-source	(n=)	Persistent neutropenia and 2nd HSCT			Cause of death					
		Persistent neutropenia	2nd HSCT	Subsequent neutropenia	Progression of fulminant primary HLH	TRM: Bacterial infection	TRM: Viral infection	TRM: Chronic GVHD	TRM: Organ failure	
MAC-BMT	(12)	0			1	1	0	1	0	
RIC-BMT	(3)	0			0	1	0	0	0	0.12
MAC-CBT	(25)	3	→2	→1[a]	1	2[a]	2	0	4[b]	
RIC-CBT	(13)	2	→2	→0	0	2	0	0	3[c]	

Persistent and subsequent neutropenia: neutrophil count did not exceed 500/uL at day 30 after HSCT or later. Second HSCT was all CBT

TRM treatment-related mortality

[a] One patient died of bacterial infection for persistent/subsequent neutropenia

[b] Three non-infectious lung complications (2 interstitial pneumonitis and 1 acute respiratory distress syndrome) and one thrombotic micro-angiopathy (TMA)

[c] Progression of pre-HSCT organ failure due to primary HLH (i.e., without fulminant HLH after HSCT)

resulted in better engraftment. Continuous complete donor chimerism was observed at 100 days after RIC–CBT in all the patients who had once achieved complete donor chimerism at the time of engraftment. Lower doses of LPAM with Flu and ALG adversely affected the engraftment even when concomitant with cyclophosphamide 50–60 mg/kg. Regarding the influence of total body irradiation (TBI), HLA incompatibility to host-versus-graft direction, and infused-cell number on engraftment, we cannot draw any conclusion, because our study contained very small number of patients.

Discussion

Primary HLH, also called FHL, is currently understood to involve genetically impaired machinery of cytotoxic granules in the T or NK cells. Primary HLH is diagnosed based on the affected proteins as follows: perforin, FHL2; Munc13-4, FHL3; syntaxin11, FHL4; and Munc18-2, FHL5 [10, 11]. Secondary HLH accompanied by Epstein–Barr virus (EBV) infection, malignancies, and autoimmune diseases was excluded from the present analysis [10]. However, there was no information on affected proteins in the JSHCT database or TRUMP system. There are borderline HLH as well as some rare well-defined syndromes (with a known impaired protein that also affects somatic cells other than T or NK cells) such as Griscelli syndrome type 2 (Rab27a), Hermansky–Pudlak syndrome type 2 (AP3β-1 subunit) and Chediak–Higashi syndrome (CHS). Moreover, it is possible that some rare congenital metabolic disorders accompanied by HPS in secondary HLH were not excluded, such as galactosialidosis and cobalamin C disease.

Our data regarding HSCT, including CBT, are similar to those of other reports [3]; however, in our series, the survival rate after CBT was slightly, but not significantly, worse than after BMT. However, all CBTs were performed under unrelated settings. The OS rate after unrelated BMT (6 MAC and 1 RIC) was no more than 42.9 ± 18.7 %, which was worse than MAC–CBT and RIC–CBT, although the difference was not significant. Unrelated CB may be an alternative source if the patient has no related donor. However, our analysis was not based on the controlled randomized prospective study, and there were some confounding factors between the groups. More than 70 % of MAC–BMT and MAC–CBT were done before 2004, and more than 70 % of RIC–CBT was done since 2005. Patients who underwent MAC might have been treated with HLH-94-based protocol [3], and patients who underwent RIC–CBT might have been treated with HLH-2004-based protocol [2]. Supportive care has also made advances during the latest decade.

Some persisting HPS activity from the primary disease did not automatically preclude HSCT [12]. However, all of the 3 patients at PS 4 for severe organ dysfunction of the liver, lungs, and/or CNS attributed to the primary disease had extremely poor outcomes in the present study. RIC–CBT might be chosen for such patients because of its safety and emergent accessibility; however, even if the primary disease was controlled after HSCT, their organ failure was irreversible and fatal during the peri-transplant period. To

Table 3 RIC–CBT regimen and engraftment

Pt#	Age at		Sex	Conditioning regimen			Neutrophil recovery	Chimerism (engraftment)	Infused cells		HLA mm for		GVHD prophylaxis	Acute GVHD	2nd HSCT	Outcome (days)
	Diag	HSCT		Flu	LPAM	Others			ANC	CD34+	GVH	HVG				
1	5 months	7 months	F	125 (low)	180 (high)	ALG45	+	Donor	10.4	4.6	0	0	CsA/MTX	0	–	420 + alive
2	12 years	15 years	F	120 (low)	140 (high)	ALG40	+	Donor	3.3	nd	1	1	Tac/MTX	III	–	1834 + alive
3	4 years	4 years	F	120 (low)	140 (high)	ALG40	+	Donor	4.5	1.1	0	0	Tac/MTX	II	–	2276 + alive
4	2 months	7 months	M	100 (low)	120 (high)	ATG8	+	Donor	nd	nd	0	0	Tac/MTX	I	–	168 + alive
5	1 month	4 months	M	180 (high)	140 (high)	CY60	+	Mix	nd	2.5	0	0	CsA/MTX	0	–	879 + alive
6	0 month	5 months	M	180 (high)	140 (high)	–	+	Failure[a]	6.8	2.0	1	1	CsA/MTX	0	No	1127 + alive
7	3 years	4 years	F	125 (low)	80 (low)	TBI (4 Gy)	–	Failure	4.3	nd	0	0	Tac/MTX	0	Yes	1367 + alive
8	4 years	5 years	M	180 (high)	70 (low)	CY60, ALG20	–	Failure	3.8	3.2	1	2	Tac/MTX	0	Yes	515 + alive
9	2 months	6 months	M	180 (high)	70 (low)	CY50, ALG20, Etp200	+	Failure[a]	23.8	nd	0	0	Tac/MTX	0	No	54 TRM[b]
10	0 month	1 year	M	180 (high)	70 (low)	CY50, ALG20, Etp200	+	Mix	19.0	nd	0	0	Tac	II	–	30 TRM[b]

The patients who lived 30 days or more after RIC–CBT are shown ($n = 10$). HLA mm: serological mismatch in HLA-A, B and DR for graft-versus-host (GVH) or host-versus-graft (HVG) direction, chimerism: donor, complete donor chimerism (donor-type WBC > 95 %) in PB; mix, mixed chimerism with 5–95 % of donor-derived WBC; failure, engraftment failure <5 %. Neutrophil recovery was defined as absolute neutrophil count <500/uL at day 30 after HSCT

Flu fludarabine, *Etp* LPAM and etoposide (mg/m^2), *ALG/ATG* anti-lymphocyte/thymocyte globulin, *CY* cyclophosphamide (mg/kg), *Pt#* patient number, *Diag* diagnosis, *nd* no data, *TBI* total body irradiation, *TRM* treatment-related mortality

[a] Gradual reduction of donor cell ratio (finally <5 %)

[b] Progression of pre-HSCT organ failure due to primary HLH (i.e., without fulminant HLH after HSCT)

improve survival, when the HPS would be resistant to the chemotherapy and immunosuppressants, patients should be treated with HSCT as early as possible before disease progression; and in such cases, unrelated CBT is superior to unrelated BMT because CB is immediately available.

In RIC–CBT group, the rate of TRM due to post-HSCT organ failure was low while that of incomplete engraftment was high compared to the MAC–CBT. Unlike TRM, patients without neutrophil recovery or donor chimerism could be rescued with a second HSCT. Incomplete donor chimerism is a major adverse event of RIC even after BMT [5]. The present study suggests that LPAM 140 mg/m^2 with Flu and ALG/ATG might be sufficiently intense for complete donor cell engraftment and that RIC–CBT might be feasible. However, these findings are limited because of the small number of patients, variable dosages, and retrospective nature of the present study. Nevertheless, our analysis warrants a prospective study for further dosage optimization. Low-dose TBI instead of ALG/ATG might also result in complete engraftment; however, there is a concern that higher rates of subsequent primary neoplasms may occur with low-dose TBI, although this has not been reported thus far. Low-dose TBI might also have some influence on fertility. For example, it is predicted that the fractionated radiation dose of 3 and 6 Gy at the age of 0–4 years results in early ovarian failure at the age of 35.1–35.6 ± 3.9 years and 22.6–24.0 ± 3.9 years, respectively [13]. In this point of view, RIC regimen for children should not include busulfan either, because busulfan is also known to cause ovarian failure [14]. Our recommended RIC of Flu, LPAM, and ALG/ATG preserved ovarian function in adolescents and young adults [15]. Therefore, it is worthwhile to investigate whether this regimen preserves children's growth and fertility potentials. ALG/ATG usage is reported to be a risk factor for the development of viral diseases such as EBV-associated PTLD [16, 17]. Furthermore, ALG has already been commercially unavailable. Therefore, optimized dosages of ATG and Flu should be investigated. Researchers in Japan recently began a regional (i.e., not nationwide) trial of RIC with Flu, LPAM, and low-dose TBI for patients undergoing CBT [18]. Some of our patients (#1, #2, #5, #6, and #8 in Table 3) will be control patients in that regional trial, and patient #7 (Table 3) will be study patient [18].

In conclusion, the eligibility criteria for allogeneic HSCT for the treatment of primary/familial HLH should not include patients with a PS of 4 and severe organ dysfunction due to a primary disease. Unrelated RIC–CBT may be an alternative HSCT if a patient has no related donor. Patients should undergo HSCT as early as possible with a well-controlled status of primary HLH after diagnosis before the disease progresses. LPAM 140 mg/m^2 with Flu and ATG/ALG might be feasible, but further dosage optimization should be performed in controlled clinical trials.

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Clinical Factors Predicting the Response of Acute Graft-versus-Host Disease to Corticosteroid Therapy: An Analysis from the GVHD Working Group of the Japan Society for Hematopoietic Cell Transplantation

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A B S T R A C T

Systemic corticosteroid therapy is recommended as a first-line treatment for acute graft-versus-host disease (GVHD). We performed a retrospective study to identify the factors affecting the response of grade II to IV acute GVHD to systemic corticosteroid therapy using the Japanese national registry data for patients who received first allogeneic hematopoietic cell transplantation with bone marrow (BM) (n = 1955), peripheral blood stem cells (PBSCs) (n = 642), or umbilical cord blood (UCB) (n = 839). Of 3436 patients, 2190 (63.7%) showed improvement of acute GVHD to first-line therapy with corticosteroids. Various factors were identified to predict corticosteroid response. Interestingly, UCB (versus HLA-matched related BM) transplantation was significantly associated with a higher probability of improvement, whereas HLA-matched unrelated BM and HLA-mismatched stem cell sources other than UCB were significantly associated with a lower probability of improvement. HLA-matched related PBSC transplantation was not significantly different from HLA-matched related BM transplantation. Patients without improvement from corticosteroid therapy had a 2.5-times higher nonrelapse mortality and a .6-times lower overall survival rate. The present study demonstrated, for the first time, a higher probability of improvement in grade II to IV acute GVHD with systemic corticosteroid therapy in patients after UCB transplantation than in those after BM and PBSC transplantation. A prospective study is warranted.

INTRODUCTION

Despite prophylactic treatment with immunosuppressive agents, acute graft-versus-host disease (GVHD) remains a major problem after allogeneic hematopoietic cell transplantation (HCT). Several studies have evaluated a variety of agents added to prednisone [1-7], but the use of prednisone or methylprednisolone alone is recommended as a standard first-line treatment for acute GVHD [8]. The response rate is approximately 40% to 60%, and patients unresponsive or resistant to corticosteroid therapy have an increased risk of mortality related to uncontrolled GVHD [2,9-16]. Some clinical factors are reported to be statistically predictive of a response to systemic corticosteroid therapy: HLA-mismatched donor transplantation, unrelated donor transplantation, combination of male recipient and female donor, early onset of GVHD, higher grade of GVHD, and liver or gut involvement of GVHD have lower response rates [2,9,10,14].

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These significant factors were identified in retrospective studies in which most or all patients underwent bone marrow (BM) transplantation. However, stem cell sources for allogeneic HCT have changed dramatically with the frequent use of peripheral blood stem cells (PBSCs) and umbilical cord blood (UCB), and no study has compared the response rates of corticosteroid therapy among stem cell sources.

To identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for patients with grade II to IV acute GVHD, a retrospective study was conducted using the national registry data on 3436 patients who received first allogeneic HCT in Japan with BM (n = 1955), PBSCs (n = 642), or UCB (n = 839).

PATIENTS AND METHODS

Patients

Clinical data for patients who received the first allogeneic HCT in Japan, achieved neutrophil engraftment (>.5 × 10^9/L), developed grade II to IV acute GVHD, and received systemic corticosteroid therapy as a first-line treatment for acute GVHD were extracted from the Transplant Registry Unified Management Program system, which is a registry of the outcomes of Japanese transplantation patients [17]. Patients who relapsed before GVHD development were excluded, as were patients who received other agents as initial therapy in addition to systemic corticosteroid therapy. This study was approved by the Data Management Committee of the Japan Society for Hematopoietic Cell Transplantation and by the ethical committee of the Nagoya University School of Medicine.

Definitions

Acute GVHD was diagnosed and graded according to established criteria [18]. Persistent nausea with histologic evidence of GVHD but no diarrhea was included as stage 1 gut GVHD. Responses of acute GVHD to corticosteroid therapy were defined as *improved* if the grade was improved without additional systemic treatment. Responses were evaluated without time limitation, and therefore were considered improved even if the GVHD was improved later than day 28 of corticosteroid therapy, although response by day 28 is proposed as the best endpoint to define need for second-line treatment [16]. Responses were also considered improved even if acute GVHD was improved and then a new immunosuppressant was added to treat chronic GVHD. Responses were defined as *stable or progressive* if the grade was unchanged or worsened after first-line corticosteroid therapy or if second-line systemic treatment for acute GVHD was added regardless of responsiveness to first-line corticosteroid therapy. Thus, all patients who received second-line treatment for acute GVHD were considered stable or progressive even if the GVHD was improved temporarily after corticosteroid therapy.

Acute myeloid leukemia in the first or second remission, acute lymphoblastic leukemia in the first remission, chronic myelogenous leukemia in the first chronic phase, and myelodysplastic syndromes with refractory anemia or refractory anemia with ringed sideroblasts were defined as *standard-risk malignancies*, and other malignant diseases were defined as *high-risk malignancies*.

BM transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-BM*, and BM transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-BM*. PBSC transplantation from serological HLA-A, B, and DR 6/6 matched related donors was defined as *MRD-PB*, and PBSC transplantation from serological HLA-A, B, and DR at least 3/6 matched, but not 6/6 matched related donors, was defined as *MMRD-PB*. For unrelated BM transplantation, all patient–donor pairs were HLA-typed to allele level for at least 3 loci (HLA-A, B, and DRB1) during the coordination process. BM transplantation from HLA-A, B, and DRB1 alleles 6/6 matched unrelated donors was defined as *MUD-BM*, and BM transplantation from HLA-A, B, and DRB1 alleles 5/6 or 4/6 matched unrelated donors was defined as *MMUD-BM*. UCB transplantation from serological HLA-A, B, and DR at least 4/6 matched donors was defined as *UCB*.

Based on the report by the Center for International Blood and Marrow Transplant Research [19], the conditioning regimens were classified as *myeloablative* if total body irradiation >8 Gy, oral busulfan ≥9 mg/kg, intravenous busulfan ≥7.2 mg/kg, or melphalan >140 mg/m^2 was included in the conditioning regimen, whereas other conditioning regimens were classified as *nonmyeloablative*.

Onset of acute GVHD was classified into 3 groups: day ≤28, day ≥29, and unknown; however, acute GVHD that occurred earlier than day 4, which might be an error at the time of registration, was classified into unknown.

Endpoints

The primary endpoint of this study was to identify the factors affecting the response to systemic corticosteroid therapy as a first-line treatment for grade II to IV acute GVHD. The secondary endpoints were to identify factors associated with nonrelapse mortality (NRM) after corticosteroid therapy and to evaluate the impact of response to corticosteroid therapy on the overall survival (OS) rate after corticosteroid therapy.

Statistical Analysis

Univariate and multivariate logistic regression analyses were used to identify factors associated with the response to corticosteroid therapy. The probability of NRM after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated on the basis of cumulative incidence curves in which relapse was treated as a competing event [20]. The probability of OS after systemic corticosteroid therapy stratified by response to corticosteroid therapy was estimated according to the Kaplan-Meier method [21]. The groups were compared using the log-rank test. Competing risk regression analysis was used to identify factors associated with NRM after corticosteroid therapy. The adjusted probability of OS after corticosteroid therapy was estimated using the Cox proportional hazards model, with consideration of other significant clinical variables in the final multivariate models [22]. *P* values were 2 sided, and $P < .05$ was considered significant. The following covariates were considered for the multivariate models: patient age, patient sex, sex mismatch between patient and donor, disease, stem cell source, cytomegalovirus serostatus, preconditioning, GVHD prophylaxis, in vivo T cell depletion, year of transplantation, onset of acute GVHD, grade of acute GVHD, organ involvement of acute GVHD, and response to systemic corticosteroid therapy (improved or stable/progressive). The data were analyzed by STATA version 12 statistical software (StataCorp, TX).

RESULTS

Patient, Transplantation, and GVHD Characteristics

A total of 3436 patients met the inclusion criteria. Patient and transplantation characteristics are shown in Table 1. Patient age at transplantation ranged from 0 to 82 years (median, 40 years); the number of patients age <18, 18 to 49, and ≥50 years was 672, 1626, and 1138, respectively. Stem cell sources were BM (n = 1955), PBSC (n = 642), and UCB (n = 839). All UCB transplantation was performed with a single unit. In vivo T cell depletion was performed in 168 (5%) patients by either antithymocyte globulin or antilymphocyte globulin. No other drugs, such as alemtuzumab, were used for in vivo T cell depletion, nor was ex vivo T cell depletion used in any patients. The year of transplantation ranged from 1984 to 2009; the majority of cases (94%) were performed in 2000 or later.

Characteristics of acute GVHD cases are shown in Table 2. The numbers of patients who developed acute GVHD at day ≤28 and day ≥29 were 2344 and 994, respectively. Of 3436 patients who received systemic corticosteroid therapy as the first-line treatment for grade II to IV acute GVHD, 2190 (63.7%) showed improvement of acute GVHD.

Factors Associated with Improvement of GVHD by Corticosteroid Therapy

MUD-BM, HLA-mismatched stem cell source other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement of acute GVHD, including gut, were significantly associated with a lower probability of improvement by corticosteroid therapy (Table 3). On the other hand, adult patient (ages 18 to 49 years) and UCB were significantly associated with a higher probability of improvement by corticosteroid therapy (Table 3). Although some factors, such as disease, cytomegalovirus serostatus, and preconditioning, were significant for corticosteroid response in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between

Table 1
Patient and Transplantation Characteristics (N = 3436)

Characteristic	Total (N = 3246)	MRD-BM/PB (n = 926)	MUD-BM + mm* (n = 1671)	UCB (n = 839)
Patient age at transplantation				
<18 yr	672 (20)	99 (11)	310 (19)	263 (31)
18 to 49 yr	1626 (47)	520 (56)	836 (50)	270 (32)
≥50 yr	1138 (33)	307 (33)	525 (31)	306 (37)
Patient sex				
Female	1393 (41)	380 (41)	668 (40)	345 (41)
Male	2043 (59)	546 (59)	1003 (60)	494 (59)
Sex mismatch between patient and donor				
Female donor to male patient	815 (24)	251 (27)	348 (21)	216 (26)
Other combinations	2525 (73)	662 (72)	1321 (79)	542 (64)
Unknown	96 (3)	13 (1)	2 (0)	81 (10)
Disease				
Standard-risk malignancies	1320 (38)	372 (40)	686 (41)	262 (31)
High-risk malignancies	1926 (57)	509 (55)	900 (54)	517 (62)
Nonmalignancies	154 (4)	40 (4)	80 (5)	34 (4)
Unknown	36 (1)	5 (1)	5 (0)	26 (3)
Stem cell source				
MRD-BM	445 (13)	445 (48)	0 (0)	0 (0)
MRD-PB	481 (14)	481 (52)	0 (0)	0 (0)
MUD-BM	783 (23)	0 (0)	783 (47)	0 (0)
UCB	839 (24)	0 (0)	0 (0)	839 (100)
MMRD-BM	155 (4)	0 (0)	155 (9)	0 (0)
MMRD-PB	161 (5)	0 (0)	161 (10)	0 (0)
MMUD-BM	572 (17)	0 (0)	572 (34)	0 (0)
Cytomegalovirus serostatus				
Negative donor to negative patient	322 (9)	53 (6)	112 (7)	159 (19)
Positive donor to negative patient	215 (6)	64 (7)	149 (9)	0 (0)
Negative donor to positive patient	899 (26)	107 (12)	290 (17)	509 (61)
Positive donor to positive patient	1541 (46)	574 (61)	960 (57)	0 (0)
Unknown	459 (13)	128 (14)	160 (10)	171 (20)
Preconditioning				
Myeloablative	2094 (61)	578 (62)	1030 (62)	486 (58)
Nonmyeloablative	1307 (38)	323 (35)	636 (38)	348 (41)
Unknown	35 (1)	25 (3)	5 (0)	5 (1)
GVHD prophylaxis				
Cyclosporine A–based	1676 (49)	800 (87)	417 (25)	459 (55)
Tacrolimus-based	1691 (49)	103 (11)	1227 (73)	361 (43)
Others	56 (2)	20 (2)	26 (2)	10 (1)
Unknown	13 (0)	3 (0)	1 (0)	9 (1)
In vivo T cell depletion				
No	3251 (95)	876 (94)	1556 (93)	819 (98)
Yes	168 (5)	34 (4)	115 (7)	19 (2)
Unknown	17 (0)	16 (2)	0 (0)	1 (0)
Year of transplantation				
1984 to 1999	200 (6)	103 (11)	63 (4)	34 (4)
2000 to 2004	721 (21)	182 (20)	221 (13)	318 (38)
2005 to 2009	2515 (73)	641 (69)	1387 (83)	487 (58)

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease.
Data presented are n (%).
* mm indicates MMRD-BM, MMRD-PB, and MMUD-BM.

onset of acute GVHD and response to corticosteroid therapy. Response rates to corticosteroid therapy in each stem cell source are summarized in Table 4.

Impact of the Response to Corticosteroid Therapy on NRM

The cumulative incidence rates of NRM after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 1. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had a significantly higher NRM compared with those who achieved improvement ($P < .0001$).

To identify factors associated with NRM after corticosteroid therapy for grade II to IV acute GVHD, competing risk regression analysis was performed. The patients with a stable or progressive response to corticosteroid therapy were approximately 2.5 times more likely to have NRM than patients with an improved response to corticosteroid therapy (Table 5). Other factors associated with significantly worse NRM included older patient age (18 to 49 years and ≥50 years), higher grades of acute GVHD (grades III and IV), and liver or multiple organ involvement including liver of acute GVHD (Table 5). Although some factors such as patient sex, disease, and preconditioning were significant for NRM in univariate analysis, they were not significant in multivariate analysis. Additional analysis in which onset of acute GVHD was modeled as a continuous variable could not detect a significant association between onset of acute GVHD and NRM.

Impact of the Response to Corticosteroid Therapy on the OS Rate

The Kaplan-Meier estimates of OS rates after systemic corticosteroid therapy for grade II to IV acute GVHD are shown in Figure 2. Patients who did not achieve improvement of acute GVHD by corticosteroid therapy had

Table 2
Acute GVHD Characteristics

Characteristic	Total (N = 3436)	MRD-BM/PB (n = 926)	MUD-BM + mm* (n = 1671)	UCB (n = 839)
Onset of acute GVHD				
Day ≤28	2344 (68)	560 (60)	1221 (73)	563 (67)
Day ≥29	994 (29)	351 (38)	434 (26)	209 (25)
Unknown	98 (3)	15 (2)	16 (1)	67 (8)
Grade of acute GVHD				
II	2049 (59)	584 (63)	973 (58)	492 (58)
III	1015 (30)	259 (28)	482 (29)	274 (33)
IV	372 (11)	83 (9)	216 (13)	73 (9)
Organ involvement				
Skin only	1110 (32)	288 (31)	579 (34)	243 (29)
Gut only	310 (9)	125 (13)	129 (8)	55 (7)
Liver only	35 (1)	8 (1)	16 (1)	11 (1)
Skin and gut, no liver	1178 (34)	316 (34)	576 (34)	286 (34)
Skin and liver, no gut	177 (5)	56 (6)	72 (4)	49 (6)
Gut and liver, no skin	87 (3)	26 (3)	42 (3)	19 (2)
Skin, gut, and liver	487 (14)	107 (12)	256 (16)	124 (15)
Unknown	52 (2)	0 (0)	1 (0)	51 (6)

GVHD indicates graft-versus-host disease; MRD-BM/PB, HLA-matched related donor bone marrow and HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood.
Data are presented as n (%).
* mm indicates HLA-mismatched related donor bone marrow, HLA-mismatched related donor peripheral blood stem cells and HLA-mismatched unrelated donor bone marrow.

a significantly lower OS rate than those who achieved improvement ($P < .0001$).

To evaluate the impact of the response to corticosteroid therapy on the OS rate, the Cox proportional hazards model was used with all of the clinical features listed in Tables 1 and 2. On univariate analysis, the OS rate was significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 2.18; 95% confidence interval, 1.97 to 2.40). After adjustment by patient age, disease, preconditioning, grade of acute GVHD, and organ involvement of acute GVHD, which were significant on univariate analysis, the OS rate

Table 3
Factors Associated with Improvement of GVHD by Corticosteroid Therapy

Factor (n)	Univariate Analysis Relative Risk* (95% CI)	P Value	Multivariate Analysis Relative Risk* (95% CI)	P Value
Patient age				
<18 yr (672)	1		1	
18 to 49 yr (1626)	1.33 (1.10 to 1.60)	.003	1.48 (1.18 to 1.85)	<.002
≥50 yr (1138)	1.06 (.88 to 1.30)	.509	1.11 (.88 to 1.40)	.385
Stem cell source				
MRD-BM (445)	1		1	
MRD-PB (481)	.66 (.50 to .87)	.004	.81 (.59 to 1.12)	.201
MUD-BM (783)	.53 (.41 to .68)	<.001	.57 (.43 to .76)	<.001
UCB (839)	.97 (.75 to 1.26)	.839	1.36 (1.01 to 1.83)	.042
MMRD-BM (155)	.26 (.18 to .39)	<.001	.37 (.24 to .57)	<.001
MMRD-PB (161)	.34 (.23 to .49)	<.001	.41 (.27 to .63)	<.001
MMUD-BM (572)	.47 (.36 to .61)	<.001	.57 (.42 to .77)	<.001
GVHD prophylaxis				
Cyclosporine A-based (1676)	1		1	
Tacrolimus-based (1691)	.80 (.69 to .92)	.002	1.02 (.82 to 1.26)	.851
Other (56)	.38 (.22 to .64)	<.001	.61 (.31 to 1.22)	.164
In vivo T cell depletion				
No (3251)	1		1	
Yes (168)	1.47 (1.08 to 2.01)	.015	1.06 (.68 to 1.65)	.787
Onset of acute GVHD				
Day ≤28 (2344)	1		1	
Day ≥29 (994)	1.20 (1.03 to 1.40)	.023	1.10 (.91 to 1.34)	.336
Grade of acute GVHD				
II (2049)	1		1	
III (1015)	.34 (.29 to .39)	<.001	.45 (.37 to .55)	<.001
IV (372)	.04 (.03 to .06)	<.001	.07 (.05 to .10)	<.001
Organ involvement				
Skin only (1110)	1		1	
Gut only (310)	.69 (.52 to .92)	.011	.91 (.66 to 1.24)	.541
Liver only (35)	.22 (.11 to .43)	<.001	.56 (.25 to 1.25)	.157
Skin and gut, no liver (1178)	.55 (.45 to .66)	<.001	.77 (.62 to .96)	.021
Skin and liver, no gut (177)	.39 (.28 to .54)	<.001	.78 (.53 to 1.15)	.214
Gut and liver, no skin (87)	.17 (.11 to .26)	<.001	.36 (.21 to .59)	<.001
Skin, gut, and liver (487)	.13 (.10 to .17)	<.001	.38 (.28 to .51)	<.001

GVHD indicates graft-versus-host disease; MRD-BM, HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; CI, confidence interval.
* Values >1.0 indicate higher probability of improvement; values <1.0 indicate lower probability.

Table 4
Response to Corticosteroid Therapy in Each Stem Cell Source

Stem Cell Source	No. of Cases	Patients with Improved Response, n (%)
MRD-BM	445	328 (73.7)
MRD-PB	481	312 (64.9)
MUD-BM	783	468 (59.8)
UCB	839	614 (73.2)
MMRD-BM	155	66 (42.9)
MMRD-PB	161	78 (48.4)
MMUD-BM	572	324 (56.6)
Total	3436	2190 (63.7)

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow.

was still significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 1.66; 95% confidence interval, 1.49 to 1.85).

DISCUSSION

The present nationwide study revealed that the response rate of grade II to IV acute GVHD to systemic corticosteroid therapy in Japanese patients was approximately 64%, which is comparable to that in Caucasian patients. In a retrospective analysis of 456 patients who were treated with methylprednisolone 2 mg/kg/day for grade II to IV acute GVHD after allogeneic BM transplantation at the Fred Hutchinson Cancer Research Center, 59% of the patients experienced a complete, partial, or mixed response [10]. In another retrospective analysis of 864 patients who were treated with prednisone 60 mg/m^2/day for grade II to IV acute GVHD after BM, PBSC, or UCB transplantation at the University of Minnesota, 65% of the patients experienced a complete, very good partial, or partial response [16].

The factors associated with poor response to corticosteroid therapy were MUD-BM, HLA-mismatched stem cell sources other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement including gut of acute GVHD (Table 3). The previous studies also found these features as risk factors for an increased treatment failure rate [9,10], suggesting that these subgroups may be targets for alternate first-line immunosuppressive therapies.

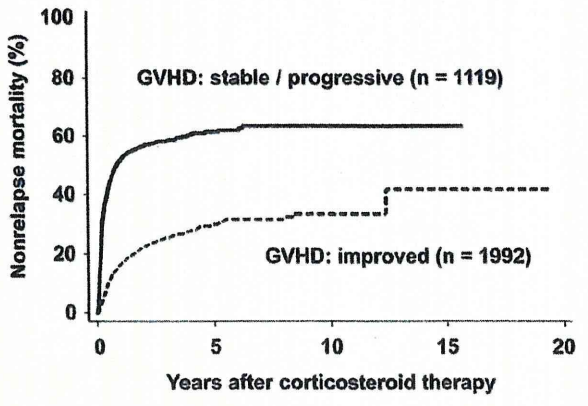

Figure 1. Nonrelapse mortality (NRM) after systemic corticosteroid therapy for patients with grade II to IV acute GVHD. Cumulative incidence rates of NRM after systemic corticosteroid therapy in patients (n = 1992) with an improved response to corticosteroid therapy (dashed line, 22.2% [95% confidence interval, 20.1% to 24.4%] at 2 years, 30.1% [27.1% to 33.0%] at 5 years, 33.5% [29.4% to 37.6%] at 10 years, and 41.8% [26.2% to 56.7%] at 15 years) and patients (n = 1119) with a stable or progressive response to corticosteroid therapy (solid line, 56.3% [53.1% to 59.5%] at 2 years, 61.4% [57.7% to 64.9%] at 5 years, 63.4% [59.2% to 67.3%] at 10 years, and 63.4% [59.2% to 67.3%] at 15 years) are shown ($P < .0001$).

On the other hand, UCB was identified as a factor associated with a higher response to first-line corticosteroid therapy in the present study (Table 3). Although several studies have demonstrated a significantly lower incidence of acute GVHD in UCB transplantation than in unrelated BM transplantation [23-29], no study has compared the response to treatment of acute GVHD between them. The present study demonstrated, for the first time, a higher response of grade II to IV acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation than in those after BM or PBSC transplantation.

Nevertheless, UCB transplantation had no impact on NRM after corticosteroid therapy in the multivariate analysis and, in fact, had higher NRM than MRD-BM transplantation in the univariate analysis (Table 5). Thus, even though there was a higher response of acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation, careful management is required for patients who suffer from grade II to IV acute GVHD after UCB transplantation, as well as those after transplantation with other stem cell sources.

Unexpectedly, adult patient (ages 18 to 49 years) was predictive of a good response to systemic corticosteroid therapy compared with child patient (age <18 years). Additional analysis was performed, and it was found that patients with grade II acute GVHD accounted for 61.4% of adult patient group, whereas 56.1% of child patient group (Fisher exact test, $P = .019$). This difference might affect the above result because severity of acute GVHD was the most significant factor associated with response to corticosteroid therapy (Table 3). Nonetheless, adult patients were likely to have higher NRM than child patients (Table 5). Our data indicate that although adult patients may be more responsive to corticosteroid therapy for acute GVHD, they have a higher risk of transplant-related toxicity than children with acute GVHD.

Despite the fact that multivariate analysis showed a significantly higher response rate to corticosteroid therapy in UCB transplantation than MRD-BM transplantation, the actual percentage was similar between UCB (73.2%) and MRD-BM (73.7%) transplantations (Table 4). Additional analysis found that patients in the age group 18 to 49 years (predictive factor of good response) accounted for only 32.2% of UCB transplantation, but constituted 58.4% of the MRD-BM population (Fisher exact test, $P < .001$) and that patients with grade II acute GVHD (predictive factor of good response) accounted for only 58.6% of UCB transplantation, but constituted 70.1% of the MRD-BM population (Fisher exact test, $P < .001$). These data suggested that the UCB population included fewer patients having predictive factors of good response to corticosteroid therapy compared with the MRD-BM population. This could explain why the actual percentage of patients with an improved response in UCB transplantation was almost the same as the percentage of patients with an improved response in MRD-BM transplantation.

Interestingly, multiorgan involvement that includes the gut was less likely to respond to first-line therapy with corticosteroids (Table 3); however, patients with liver involvement are more likely to have higher NRM (Table 5). Further study is required to elucidate the mechanisms of the difference in the effect of gut and liver GVHD on

Table 5
Factors Associated with Nonrelapse Mortality after Corticosteroid Therapy

Factor (n)	Univariate Analysis Hazard Ratio* (95% CI)	P Value	Multivariate Analysis Hazard Ratio* (95% CI)	P Value
Patient age				
<18 yr (554)	1		1	
18 to 49 yr (1503)	1.50 (1.21 to 1.85)	<.001	1.72 (1.38 to 2.14)	<.001
≥50 yr (1054)	2.74 (2.22 to 3.38)	<.001	3.34 (2.67 to 4.17)	<.001
Stem cell source				
MRD-BM (402)	1		1	
MRD-PB (447)	1.43 (1.11 to 1.83)	.005	.88 (.68 to 1.15)	.344
MUD-BM (726)	1.40 (1.11 to 1.77)	.004	1.02 (.80 to 1.30)	.866
UCB (720)	1.35 (1.06 to 1.71)	.014	1.15 (.90 to 1.48)	.265
MMRD-BM (141)	1.63 (1.16 to 2.28)	.005	1.15 (.82 to 1.62)	.415
MMRD-PB (153)	1.74 (1.26 to 2.39)	.001	.97 (.69 to 1.37)	.882
MMUD-BM (522)	1.79 (1.41 to 2.27)	<.001	1.25 (.97 to 1.60)	.082
GVHD prophylaxis				
Cyclosporine A-based (1528)	1			
Tacrolimus-based (1520)	1.06 (.94 to 1.21)	.332		
Other (50)	1.28 (.81 to 2.04)	.296		
In vivo T cell depletion				
No (3004)	1			
Yes (91)	.98 (.66 to 1.44)	.919		
Onset of acute GVHD				
Day ≤28 (2212)	1			
Day ≥29 (899)	1.05 (.92 to 1.20)	.476		
Grade of acute GVHD				
II (1864)	1		1	
III (917)	2.21 (1.92 to 2.56)	<.001	1.56 (1.31 to 1.86)	<.001
IV (330)	7.93 (6.67 to 9.43)	<.001	3.53 (2.84 to 4.38)	<.001
Organ involvement				
Skin only (1010)	1		1	
Gut only (266)	1.11 (.84 to 1.47)	.448	.80 (.59 to 1.08)	.139
Liver only (28)	4.11 (2.20 to 7.69)	<.001	2.22 (1.19 to 4.16)	.013
Skin and gut, no liver (1083)	1.27 (1.06 to 1.51)	.008	.97 (.79 to 1.18)	.753
Skin and liver, no gut (160)	2.42 (1.83 to 3.21)	<.001	1.54 (1.13 to 2.08)	.006
Gut and liver, no skin (75)	3.64 (2.57 to 5.16)	<.001	1.88 (1.29 to 2.73)	.001
Skin, gut, and liver (448)	4.82 (4.03 to 5.77)	<.001	2.07 (1.64 to 2.62)	<.001
Response to systemic corticosteroid therapy				
Improved (1992)	1		1	
Stable/progressive (1119)	3.63 (3.20 to 4.12)	<.001	2.45 (2.14 to 2.82)	<.001

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease; CI, confidence interval.
* Values >1.0 indicate higher probability of non relapse mortality; values <1.0 indicate lower probability.

transplantation outcome. Nevertheless, lack of response to initial therapy is an important risk factor in predicting high NRM in patients with grade II to IV acute GVHD (Table 5).

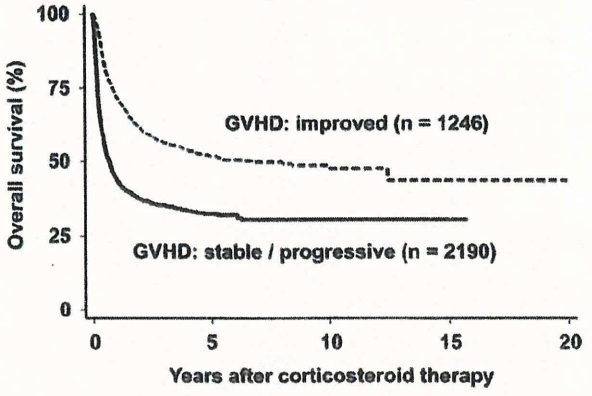

Figure 2. Overall survival (OS) for patients with grade II to IV acute GVHD. OS for patients (n = 2190) with an improved response (dashed line; 61.3% [95% confidence interval, 59.0% to 63.5%] at 2 years, 51.9% [49.2% to 54.5%] at 5 years, 47.8% [44.0% to 51.5%] at 10 years, and 43.8% [35.5% to 51.8%] at 15 years) and OS for patients (n = 1246) with a stable or progressive response (solid line; 37.4% [34.6% to 40.3%] at 2 years, 32.5% [29.5% to 35.6%] at 5 years, 30.6% [27.3% to 34.1%] at 10 years, and 30.6% [27.3% to 34.1%] at 15 years) are shown ($P < .0001$).

The patients who did not achieve improvement of acute GVHD by corticosteroid therapy had approximately 2.5-times higher NRM and approximately .6-times lower OS rates. It is well known that the incidence of acute GVHD in Japanese patients is lower than that in Caucasian patients [30,31]. However, the present data clearly demonstrate that, if the systemic corticosteroid therapy is ineffective, even Japanese patients cannot achieve a satisfactory survival rate. Another important message of this study is that the establishment of second-line treatment for corticosteroid-refractory acute GVHD is required for not only Caucasian, but also for Japanese patients.

This study had several limitations. First, the sort and dose of corticosteroids are not collected in the Japan Society for Hematopoietic Cell Transplantation database. In patients with grade II to IV acute GVHD, initial treatment with prednisone-equivalent steroid doses higher than 2.5 mg/kg has not been shown to provide better outcomes [32], although in patients with grade II acute GVHD, lower-dose initial treatment at 1.0 mg/kg has not been shown to provide worse outcomes [33]. The intensity of corticosteroid therapy may differ by each transplantation team or each patient, as shown by a survey in Europe [34], and this information may give us additional findings. Second, criteria for improvement, or for stable or progressive acute GVHD, had been previously defined in the