The incidence of cGVHD was retrospectively evaluated by using the NIH consensus criteria [18]. Patients who had at least one "diagnostic" clinical sign or at least one "distinctive" manifestation, confirmed by relevant laboratory tests or histologic examination, were defined as having cGVHD if other possible diagnoses were excluded. Subclassification of cGVHD into "classic cGVHD" and "overlap syndrome" was strictly according to the NIH criteria. If patients had any features of aGVHD along with classic cGVHD, they were classified as having an overlap syndrome. The severity of cGVHD was assessed at its onset and at maximal clinical worsening and graded into "mild", "moderate", and "severe" categories according to the global scoring system defined by the NIH criteria. Treatment of cGVHD was variable, but followed some general principles; patients with isolated mouth, ocular, or localized skin cGVHD were treated only with topical therapy, while patients with more symptomatic cGVHD were treated with systemic immunosuppressive agents such as prednisolone at a dose of 0.5–1.0 mg/kg per day combined with calcineurin inhibitors. Although the duration and dosing of those agents were not standardized, patients typically received treatment until all symptoms of cGVHD were resolved or stabilized. Patients with less severe symptoms were often treated with peroral low-dose prednisolone at a dose of less than 0.5 mg/kg per day.

2.4 Statistical analysis

Descriptive statistics were used to summarize variables related to patient and transplant characteristics. Comparisons among the groups were performed by use of extended Fisher exact test for categorical variables and Wilcoxon–Mann–Whitney test for continuous variables. The primary endpoint of the study was cGSS, which is defined as the time from the day of diagnosis of cGVHD to the day of death in the absence of relapse or secondary malignancy, among patients who developed NIH cGVHD stratified by its subcategory or severity at onset. The probabilities of cGSS were estimated according to the Kaplan–Meier method, and univariable comparison between groups was made using the log-rank test. Patients who were alive without recurrent or secondary malignancy were censored at their last follow-up visit and those who experienced recurrent or secondary malignancy were censored at the time of its diagnosis. The time to discontinuation of IST was defined among patients who received systemic IST for the treatment of NIH cGVHD as the time from the day of diagnosis of cGVHD to the day of withdrawal of systemic IST. NRM was defined among all patients included in the study as rates of death without evidence of primary disease recurrence. The incidence rates of IST withdrawal and those of NRM were estimated with the use of the cumulative incidence method to accommodate the following competing events [31]: the onset of recurrent or secondary malignancy and death from any cause for IST withdrawal, and the recurrent primary disease for NRM. Cox proportional-hazards regression models were used to evaluate variables potentially associated with cGSS, while competing risks regression models were used to evaluate variables potentially associated with IST withdrawal and NRM [32]. The variables included in the analysis were as follows: patient age, donor–recipient sex combination, disease status at the time of transplantation, donor–recipient HLA compatibility, stem cell sources, type of conditioning regimens, grades of prior aGVHD (grades 0–1 vs. grades 2–4), subcategory of NIH cGVHD (classic cGVHD vs. overlap syndrome), global severity of NIH cGVHD at onset (mild to moderate vs. severe), platelet counts, eosinophil counts, and administration of systemic corticosteroids at the onset of cGVHD. In the analysis to evaluate the impact of the presence of each NIH cGVHD subtype on NRM for the entire cohort of patients in the study, development of each subtype of cGVHD was treated as a time-dependent covariate under the assumption that a patient who developed moderate or severe cGVHD could not revert to less severe cGVHD and that classic cGVHD and overlap syndrome could not switch to each other [33]. Factors having two-sided P values less than 0.1 for association with outcome were included in multivariable model using a forward and backward stepwise method with a predetermined risk of 0.1. Two-sided P values <0.05 were considered to be statistically significant. All analyses were performed using STATA version 11 (College Station, TX, USA) according to patient information available as of 1 July 2009.

3 Results

3.1 Patient characteristics

Table 1 shows the characteristics of the 211 patients included in the study; they had a median age of 46 years, included 113 males and 98 females, and underwent transplantation for malignant hematologic neoplasms in most cases. The number of patients who received bone marrow, peripheral blood, and cord blood unit was 152 (72%), 44 (21%), and 15 (7%), respectively. After a median follow-up of 37.2 months (range 3.3–111.6), a total of 96 patients (45%) developed manifestations of cGVHD that met the NIH consensus criteria. There was no statistically significant difference in background characteristics between patients who developed NIH cGVHD and those who did not, except that the former group included higher proportion of patients with a history of antecedent grade II–IV aGVHD.

Table 1 Patient and transplantation characteristics

Characteristic	All patients ($n = 211$)	NIH cGVHD		
		Absent ($n = 115$)	Present ($n = 96$)	P value
Median patient age, years (range)	46 (17–69)	46 (19–69)	47 (17–67)	0.90
Donor/recipient sex combination, *n* (%)				0.17
Male/male	66 (31)	41 (35)	25 (26)	
Male/female	42 (20)	21 (18)	21 (22)	
Female/female	56 (27)	33 (29)	23 (24)	
Female/male	47 (22)	20 (17)	27 (28)	
Diagnosis, *n* (%)				0.59
Myeloid neoplasms	113 (54)	65 (57)	48 (50)	
Precursor lymphoid neoplasms	31 (15)	17 (15)	14 (15)	
Mature lymphoid neoplasms	61 (29)	29 (25)	32 (33)	
Aplastic anemia	6 (3)	4 (3)	2 (2)	
Disease status at transplant, *n* (%)				0.41
Standard risk	105 (50)	54 (47)	51 (53)	
High risk	106 (50)	61 (53)	45 (47)	
Donor type[a], *n* (%)				0.71
HLA-matched related	83 (39)	45 (39)	38 (40)	
HLA-mismatched related	23 (11)	12 (10)	11 (11)	
HLA-matched unrelated	89 (42)	47 (41)	42 (44)	
HLA-mismatched unrelated	16 (8)	11 (10)	5 (5)	
Donor/recipient HLA compatibility[a], *n* (%)				0.59
Matched	172 (82)	92 (80)	80 (83)	
Mismatched	39 (18)	23 (20)	16 (17)	
Stem cell source, *n* (%)				0.30
Bone marrow	152 (72)	85 (74)	67 (70)	
Peripheral blood	44 (21)	20 (17)	24 (25)	
Cord blood	15 (7)	10 (9)	5 (5)	
Conditioning regimen, *n* (%)				0.55
Myeloablative with TBI	113 (54)	64 (56)	49 (51)	
Myeloablative without TBI	15 (7)	10 (9)	5 (5)	
Reduced intensity with TBI	65 (31)	33 (29)	32 (33)	
Reduced intensity without TBI	18 (9)	8 (7)	10 (10)	
GVHD prophylaxis, *n* (%)				0.73
Tacrolimus based	169 (80)	91 (79)	78 (81)	
Cyclosporine based	42 (20)	24 (21)	18 (19)	
Prior aGVHD, *n* (%)				0.048
Grade 0–1	117 (55)	70 (61)	47 (49)	
Grade 2	72 (34)	38 (33)	34 (35)	
Grade 3–4	22 (10)	7 (6)	15 (16)	
Median months (range) after transplantation[b]	37.2 (3.3–111.6)	35.6 (3.3–111.6)	40.6 (4.0–105.3)	0.14

cGVHD chronic graft-versus-host disease, *aGVHD* acute graft-versus-host disease, *TBI* total-body irradiation

[a] HLA matching was defined by 2-digit compatibility at HLA-A, -B, and -DRB1 loci

[b] Median follow-up months among patients who were alive at the time of last follow-up

Table 2 summarizes the characteristics of 96 patients who developed NIH cGVHD according to its subcategory; 77 (80%) developed "classic cGVHD" and 19 (20%) developed "overlap syndrome". A total of 31 (40%) patients with classic GVHD and 18 (95%) with overlap syndrome had a prior history of grade II–IV aGVHD. The

median time from transplantation to the onset of cGVHD in patients with overlap syndrome was shorter compared to patients with classic cGVHD (4.1 vs. 7.1 months, $P < 0.001$). All patients with overlap syndrome were graded as having moderate or severe cGVHD, whereas the proportion of patients who developed severe cGVHD was similar between patients with classic cGVHD and those with overlap syndrome. Proportions of patients with platelet counts less than $100 \times 10^3/\mu L$, eosinophil counts less than $500/\mu L$, and ongoing systemic corticosteroid treatment at the onset of cGVHD were higher among patients who developed overlap syndrome compared with those who developed classic cGVHD.

3.2 Chronic GVHD-specific survival

Of the 96 patients who developed NIH cGVHD, recurrent or secondary malignant neoplasm occurred in 27 patients and death due to any cause occurred in 31 patients. The respective 3-year probabilities of cGSS among patients who developed classic cGVHD and overlap syndrome were 88 and 70% ($P = 0.060$) (Fig. 1a), while those among subgroups of patients graded to have mild, moderate, and severe cGVHD at onset were 100, 86, and 69% (mild to moderate vs. severe, $P = 0.034$) (Fig. 1b). Table 3 shows the results of univariable and multivariable analyses for factors potentially associated with cGSS among the patients who developed NIH cGVHD. In univariable analysis, the presence of severe cGVHD and thrombocytopenia at cGVHD onset were significantly associated with lower cGSS, whereas the presence of an overlap syndrome and high-risk malignant disease tended to be associated with lower cGSS. In multivariable analysis, the presence of thrombocytopenia at cGVHD onset was the only significant factor that adversely affected cGSS [hazard ratio (HR) for mortality = 4.05, 95% confidence interval (CI) = 1.35–12.1, $P = 0.013$], although patients with severe cGVHD (HR = 2.58, 95% CI = 0.90–7.39, $P = 0.077$) or those with high-risk underlying disease (HR = 2.75, 95% CI = 0.86–8.80, $P = 0.088$) also had a trend toward lower cGSS.

3.3 Duration of systemic immunosuppressive treatment

A total of 81 patients received systemic immunosuppressive agents for the treatment of NIH cGVHD. In this group of patients, the cumulative incidence of withdrawal of systemic IST was 40% (95% CI = 29–51%) at 3 years after the onset of cGVHD, while the cumulative incidence of the competing risks of death or recurrent/secondary malignancy during systemic IST was 42% (95% CI = 32–55%) (Fig. 2). In univariable analysis, no significant association was found between discontinuation of IST and subcategory or global severity of NIH cGVHD (overlap syndrome vs. classic cGVHD, HR for IST withdrawal = 0.51, 95% CI = 0.20–1.31, $P = 0.16$; severe vs. mild to moderate, HR = 0.90, 95% CI = 0.42–1.96, $P = 0.80$). Multivariable analysis revealed two factors significantly associated with prolonged administration of systemic IST; high-risk primary disease (HR = 0.39, 95% CI = 0.19–0.77, $P = 0.007$) and the ongoing use of systemic corticosteroids at the onset of cGVHD (HR = 0.40, 95% CI = 0.19–0.84, $P = 0.015$).

3.4 Non-relapse mortality

Death from non-relapse causes occurred in 16 (17%) of 96 patients who developed NIH cGVHD and in 10 (9%) of 115 patients who did not. In a multivariable analysis of the entire series of 211 patients, treating the subcategory or peak severity of NIH cGVHD as a time-dependent covariate, development of the overlap syndrome or severe cGVHD was significantly associated with higher risk of NRM compared to the absence of cGVHD (overlap syndrome vs. no cGVHD, HR = 3.90, 95% CI = 1.32–11.6, $P = 0.014$; severe cGVHD vs. no cGVHD, HR = 6.21, 95% CI = 2.25–17.1, $P < 0.001$). Development of classic cGVHD or mild-to-moderate cGVHD was not significantly associated with higher risk of NRM when compared with the absence of NIH cGVHD (classic cGVHD vs. no cGVHD, HR for mortality = 1.39, 95% CI = 0.55–3.53, $P = 0.49$; mild-to-moderate cGVHD vs. no cGVHD, HR = 2.25, 95% CI = 0.62–8.18, $P = 0.22$).

4 Discussion

In the present study, we evaluated the clinical significance of subcategory and severity of NIH cGVHD in terms of their influences on cGSS, discontinuation of IST, and NRM using a retrospective cohort of patients who underwent allo-HCT for hematologic disorders. In univariable analysis, patients with overlap syndrome tended to have a lower probability of cGSS than those with classic cGVHD, while patients who developed severe cGVHD had significantly worse cGSS compared with those who developed mild-to-moderate cGVHD. Although such differences in cGSS according to NIH cGVHD subtypes did not reach statistical significance by multivariable analysis, patients who developed overlap syndrome or severe NIH cGVHD had a significantly higher NRM than those who did not develop any manifestation of NIH cGVHD. These results suggest that both subcategory and global severity of NIH cGVHD might be useful for evaluating the risk of GVHD-associated mortality in patients diagnosed to have cGVHD by the NIH criteria. In

Table 2 Characteristics of chronic GVHD according to subcategory defined by the National Institutes of Health criteria

Characteristics	Total (n = 96)	NIH cGVHD subcategory		
		Classic cGVHD (n = 77)	Overlap syndrome (n = 19)	P value
Median months (range) to onset of cGVHD	6.7 (2.1–29.9)	7.1 (2.7–29.9)	4.1 (2.1–20.7)	<0.001
Involved organs or sites[a], n (%)[b]				0.92
Skin	55 (57)	40 (52)	15 (79)	
Mouth	69 (72)	56 (73)	13 (68)	
Eyes	29 (30)	23 (30)	6 (32)	
Gastrointestinal tract	34 (35)	25 (32)	9 (47)	
Liver	76 (79)	61 (79)	15 (79)	
Lungs	12 (12)	9 (12)	3 (16)	
Joints and fascia	4 (4)	3 (4)	1 (5)	
Genital tract	2 (2)	2 (3)	0 (0)	
Number of involved organs or sites[a], n (%)				0.14
1	7 (7)	7 (9)	0 (0)	
2	27 (28)	24 (31)	3 (16)	
3 or more	62 (65)	46 (60)	16 (84)	
Maximum score of involved organs[a], n (%)				0.18
Score 1	22 (23)	20 (26)	2 (11)	
Score 2 (other than lungs)	26 (27)	18 (62)	8 (42)	
Score 2 (lungs)	6 (6)	4 (5)	2 (11)	
Score 3	42 (44)	35 (45)	7 (37)	
Severity at onset, n (%)				0.023
Mild	20 (21)	20 (26)	0 (0)	
Moderate	53 (55)	39 (51)	14 (74)	
Severe	23 (24)	18 (23)	5 (26)	
Severity at peak, n (%)				0.17
Mild	12 (13)	12 (16)	0 (0)	
Moderate	39 (41)	29 (38)	10 (53)	
Severe	45 (47)	36 (47)	9 (47)	
Platelet count at cGVHD onset, n (%)				0.002
100 × 10^3/μL or more	65 (68)	58 (75)	7 (37)	
Less than 100 × 10^3/μL	31 (32)	19 (25)	12 (63)	
Eosinophil count at cGVHD onset, n (%)				0.010
Less than 500/μL	68 (71)	50 (65)	18 (95)	
500/μL or more	28 (29)	27 (35)	1 (5)	
Systemic corticosteroids at cGVHD onset, n (%)				<0.001
Not received	63 (66)	61 (79)	2 (11)	
Received	33 (34)	16 (21)	17 (89)	

cGVHD chronic graft-versus-host disease

[a] Data evaluated at peak clinical worsening are shown

[b] The sum of the number per involved site is not equal to the number of evaluable patients, because the involvement of more than one organ can occur in a single patient. Accordingly, the sum of percentage among the total number of patients does not equal to one hundred

contrast, duration of IST was neither affected by NIH cGVHD subcategory nor by its severity.

While cGSS has been frequently used as a study endpoint to describe the mortality attributable to cGVHD-associated organ dysfunction, there have been no established early surrogates that help to guide the clinical management of patients with evidence of ongoing cGVHD. Given that the historic limited/extensive grading system is not a useful predictor for the severity of organ involvement in terms of mortality risk, several studies have attempted to develop

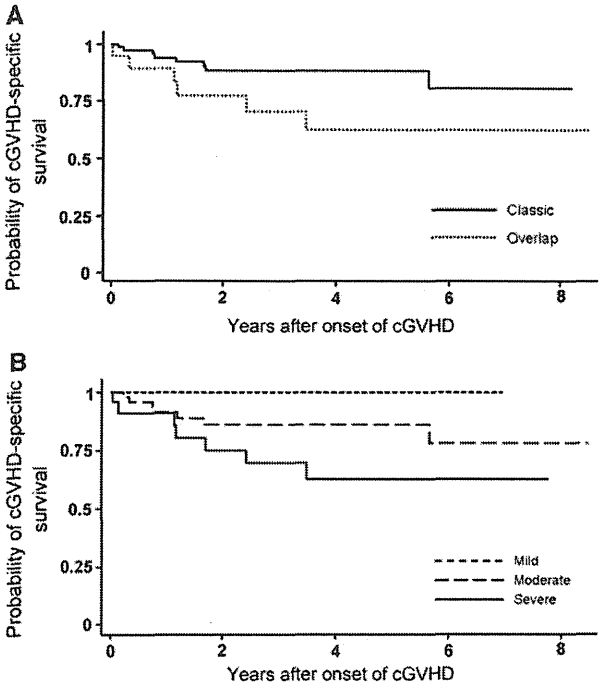

Fig. 1 Chronic GVHD-specific survival in patients with chronic GVHD diagnosed by the NIH consensus criteria. **a** Probability of chronic GVHD-specific survival (cGSS) among patients who developed classic chronic GVHD (*solid line*) and overlap syndrome (*dotted line*). **b** Probability of cGSS among patients who developed mild (*short dashed line*), moderate (*long dashed line*), and severe chronic GVHD (*solid line*)

improved grading scales for established cGVHD. A retrospective analysis of data on HLA-identical sibling transplantation reported to the International Bone Marrow Transplant Registry identified five variables independently associated with worse survival of those who developed historic cGVHD: low Karnofsky performance status at cGVHD diagnosis (<80), chronic diarrhea, weight loss, presence of cutaneous manifestation, and lack of oral involvement [15]. The Seattle group also proposed a revised classification for distinguishing limited and extensive cGVHD by the use of 16 clinical criteria [16]. Although these new classifications do not clearly discriminate between cGVHD and delayed onset GVHD with features resembling aGVHD, they have been shown to be at least useful for identifying patients at higher risk of NRM. Future studies are strongly warranted to compare the prognostic values of NIH cGVHD subcategories with those determined by other cGVHD grading system [21].

So far, several groups have reported the prognostic relevance of cGVHD severity graded by the NIH criteria and consistently found the inferior survival of patients with severe cGVHD [20–23], although such association was not observed in one earlier study [19]. While only a few of these studies focused on the significance of distinction between "overlap syndrome" and "classic cGVHD", our study revealed a trend toward worse survival in patients with overlap syndrome compared to those with classic GVHD, as was recently reported by Kim et al. [23]. In the present study, patients with overlap syndrome had a significantly shorter median time to the development of cGVHD than patients with classic cGVHD and were more likely to receive corticosteroid treatment for prior aGVHD at the onset of cGVHD. Intriguingly, these observations were very similar to the findings by Arora et al. [22], who reported that most of patients with overlap syndrome had a history of prior aGVHD and a progressive cGVHD onset, although they did not observe worse survival of this subgroup of patients compared to those with classic cGVHD. Given that nearly all patients who developed overlap syndrome had a prior history of aGVHD in our study cohort, NIH overlap syndrome in most instances could be considered as a flare of preexisting aGVHD, concomitant with development of classic cGVHD. In this context, it is important to note that early flare of cGVHD or early treatment change for exacerbation of cGVHD has been reported to be associated with increased NRM and inferior cGSS [34, 35]. It is also of note that a significantly higher proportion of patients with overlap syndrome had thrombocytopenia less than $100 \times 10^3/\mu L$ at cGVHD onset in our study. Since the progressive cGVHD onset and the presence of thrombocytopenia were consistently associated with an increased NRM across various studies [16, 36], more effective management of patients with overlap syndrome and thrombocytopenia might be needed.

Duration of systemic immunosuppressive therapy is suggested to be a useful surrogate endpoint to evaluate the response to specific treatment for cGVHD [26]. Although we could not find significant association of NIH cGVHD subtypes with duration of systemic IST, patients who had been given ongoing systemic corticosteroids at the onset of cGVHD were found to receive significantly prolonged systemic IST in multivariable analysis, consistent with the findings of Vigorito et al. [37]. In our study, the duration of systemic IST was also prolonged in patients who had high-risk underlying disease compared with those who had standard-risk disease. If the activity of cGVHD were likely to worsen in the high-risk subgroup of patients, one possible explanation might be the preference of physicians to taper systemic IST faster for patients at higher risk of relapse.

The present study, however, has several limitations; the retrospective study design, small cohort size, recording bias, and heterogeneity of underlying diseases and transplantation procedures might substantially influence the results. In addition, diagnostic cGVHD manifestations of affected organs or sites might have originated from other causes, including drug reactions, infection, and

Table 3 Univariable and multivariable analysis of factors potentially associated with chronic GVHD-specific survival among patients who developed chronic GVHD defined by the National Institutes of Health criteria

Variable	*n* (%)	Univariable analysis		Multivariable analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Patient age					
Less than 50 years	51 (53)	1.00		–	
50 years or more	45 (47)	1.40 (0.49–4.05)	0.53	–	
Donor/recipient sex combination					
Other than female/male	69 (72)	1.00		–	
Female/male	27 (28)	1.03 (0.32–3.28)	0.97	–	
Disease status at transplant					
Standard risk	51 (53)	1.00		1.00	
High risk	45 (47)	3.03 (0.95–9.68)	0.061	2.75 (0.86–8.80)	0.088
Donor/recipient HLA compatibility					
Matched	80 (83)	1.00		–	
Mismatched	16 (17)	0.33 (0.04–2.53)	0.29	–	
Conditioning regimen					
Myeloablative intensity	54 (56)	1.00		–	
Reduced intensity	42 (44)	1.04 (0.36–3.00)	0.95	–	
Stem cell source					
Bone marrow	67 (70)	1.00		–	
Peripheral blood	24 (25)	2.07 (0.69–6.19)	0.19	–	
Cord blood	5 (5)	1.63 (0.57–4.68)	0.37	–	
Prior aGVHD					
Grade 0–1	47 (49)	1.00		–	
Grade 2–4	49 (51)	1.16 (0.40–3.37)	0.78	–	
Subcategory of cGVHD					
Classic cGVHD	77 (80)	1.00		–	
Overlap syndrome	19 (20)	2.76 (0.96–7.97)	0.060	–	
Severity of cGVHD at onset					
Mild to moderate	73 (76)	1.00		1.00	
Severe	23 (24)	3.10 (1.09–8.86)	0.034	2.58 (0.90–7.39)	0.077
Platelet count at cGVHD onset					
$100 \times 10^3/\mu L$ or more	65 (68)	1.00		1.00	
Less than $100 \times 10^3/\mu L$	31 (32)	4.19 (1.40–12.5)	0.010	4.05 (1.35–12.1)	0.013
Eosinophil count at cGVHD onset					
Less than 500/μL	68 (71)	1.00		–	
500/μL or more	28 (29)	0.90 (0.28–2.88)	0.86	–	
Systemic corticosteroids at cGVHD onset					
Not received	63 (66)	1.00		–	
Received	33 (34)	1.74 (0.61–4.97)	0.30	–	

CI confidence interval, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease

comorbidity before transplantation. Furthermore, genital tract involvement might be underestimated because female patients do not always report about their genital symptoms to physicians.

In conclusion, our present study suggests that both the subcategory and global severity of cGVHD proposed by NIH consensus criteria have effects on cGSS and the risk of NRM among patients who develop NIH cGVHD. Future prospective studies are warranted to more precisely characterize the clinical significance of the subcategory and severity of cGVHD evaluated by the NIH consensus criteria.

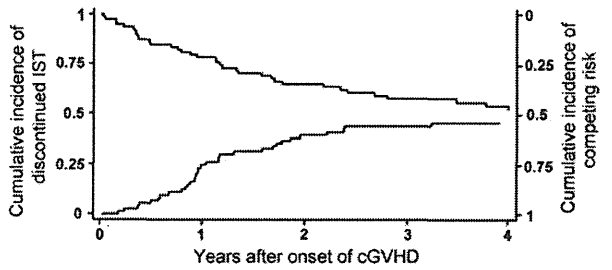

Fig. 2 Cumulative incidence of discontinued systemic immunosuppressive treatment. The *lower curve* shows the cumulative incidence of discontinued systemic immunosuppressive treatment (IST) in the absence of death, recurrent primary disease, or secondary malignancy among 81 patients who developed NIH cGVHD and received systemic IST (*left-hand scale*). The *upper curve* shows the competing risks of death or recurrent/secondary malignancy during systemic IST (*right-hand scale*). At the onset of cGVHD, 69 patients had been already given ongoing systemic IST consisting of calcineurin inhibitors alone ($n = 36$), calcineurin inhibitors plus corticosteroids ($n = 27$), corticosteroids alone ($n = 4$), or corticosteroids plus mycophenolate mofetil ($n = 2$)

Acknowledgments The authors are grateful to Rie Goi and Mika Kobayashi, for their expert data management and secretarial assistance, and all the staff of our transplant team for their dedicated care of the patients and donors.

Conflict of interest The authors have no conflict of interest to declare.

References

1. Lee S, Flowers M. Recognizing and managing chronic graft-versus-host disease. Hematology Am Soc Hematol Educ Program. 2008:134–41.
2. Atkinson K, Horowitz M, Gale R, et al. Risk factors for chronic graft-versus-host disease after HLA-identical sibling bone marrow transplantation. Blood. 1990;75:2459–64.
3. Higman M, Vogelsang G. Chronic graft versus host disease. Br J Haematol. 2004;125:435–54.
4. Wagner J, Flowers M, Longton G, Storb R, Schubert M, Sullivan K. The development of chronic graft-versus-host disease: an analysis of screening studies and the impact of corticosteroid use at 100 days after transplantation. Bone Marrow Transplant. 1998;22:139–46.
5. Przepiorka D, Anderlini P, Saliba R, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation. Blood. 2001;98:1695–700.
6. Carlens S, Ringdén O, Remberger M, et al. Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. Bone Marrow Transplant. 1998;22:755–61.
7. Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin J. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin Oncol. 2001;19:3685–91.
8. Mohty M, Kuentz M, Michallet M, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. Blood. 2002;100:3128–34.
9. Randolph S, Gooley T, Warren E, Appelbaum F, Riddell S. Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. Blood. 2004;103:347–52.
10. Atsuta Y, Suzuki R, Yamamoto K, et al. Risk and prognostic factors for Japanese patients with chronic graft-versus-host disease after bone marrow transplantation. Bone Marrow Transplant. 2006;37:289–96.
11. McClune B, Weisdorf D, Pedersen T, et al. Effect of age on outcome of reduced-intensity hematopoietic cell transplantation for older patients with acute myeloid leukemia in first complete remission or with myelodysplastic syndrome. J Clin Oncol. 2010;28:1878–87.
12. Mielcarek M, Martin P, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood. 2003;102:756–62.
13. Mielcarek M, Burroughs L, Leisenring W, et al. Prognostic relevance of 'early-onset' graft-versus-host disease following nonmyeloablative haematopoietic cell transplantation. Br J Haematol. 2005;129:381–91.
14. Shulman H, Sullivan K, Weiden P, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69:204–17.
15. Lee S, Klein J, Barrett A, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. Blood. 2002;100:406–14.
16. Lee SJ, Vogelsang G, Flowers MED. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9:215–33.
17. Atkinson K, Horowitz M, Gale R, Lee M, Rimm A, Bortin M. Consensus among bone marrow transplanters for diagnosis, grading and treatment of chronic graft-versus-host disease. Committee of the International Bone Marrow Transplant Registry. Bone Marrow Transplant. 1989;4:247–54.
18. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11:945–56.
19. Jagasia M, Giglia J, Chinratanalab W, et al. Incidence and outcome of chronic graft-versus-host disease using National Institutes of Health consensus criteria. Biol Blood Marrow Transplant. 2007;13:1207–15.
20. Pérez-Simón J, Encinas C, Silva F, et al. Prognostic factors of chronic graft-versus-host disease following allogeneic peripheral blood stem cell transplantation: the National Institutes Health scale plus the type of onset can predict survival rates and the duration of immunosuppressive therapy. Biol Blood Marrow Transplant. 2008;14:1163–71.
21. Cho B, Min C, Eom K, et al. Feasibility of NIH consensus criteria for chronic graft-versus-host disease. Leukemia. 2009;23:78–84.
22. Arora M, Nagaraj S, Witte J, et al. New classification of chronic GVHD: added clarity from the consensus diagnoses. Bone Marrow Transplant. 2009;43:149–53.
23. Kim D, Lee J, Kim S, et al. Reevaluation of the National Institutes of Health criteria for classification and scoring of chronic GVHD. Bone Marrow Transplant. 2010;45:1174–80.
24. Flowers MED, Parker PM, Johnston LJ, et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. Blood. 2002; 100:415–9.
25. Stewart B, Storer B, Storek J, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. Blood. 2004;104:3501–6.
26. Martin P, Weisdorf D, Przepiorka D, et al. National Institutes of Health Consensus Development Project on Criteria for clinical

trials in chronic graft-versus-host disease: VI. Design of Clinical Trials Working Group report. Biol Blood Marrow Transplant. 2006;12:491–505.
27. Kitawaki T, Kadowaki N, Ishikawa T, Ichinohe T, Uchiyama T. Compromised recovery of natural interferon-alpha/beta-producing cells after allogeneic hematopoietic stem cell transplantation complicated by acute graft-versus-host disease and glucocorticoid administration. Bone Marrow Transplant. 2003;32:187–94.
28. Mizumoto C, Kanda J, Ichinohe T, et al. Mycophenolate mofetil combined with tacrolimus and minidose methotrexate after unrelated donor bone marrow transplantation with reduced-intensity conditioning. Int J Hematol. 2009;89:538–45.
29. Kanda J, Mizumoto C, Kawabata H, et al. Clinical significance of serum hepcidin levels on early infectious complications in allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15:956–62.
30. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–8.
31. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695–706.
32. Kim H. Cumulative incidence in competing risks data and competing risks regression analysis. Clin Cancer Res. 2007; 13:559–65.
33. Cortese G, Andersen P. Competing risks and time-dependent covariates. Biom J. 2010;52:138–58.
34. Kim DH, Sohn SK, Baek JH, et al. Time to first flare-up episode of GVHD can stratify patients according to their prognosis during clinical course of progressive- or quiescent-type chronic GVHD. Bone Marrow Transplant. 2007;40:779–84.
35. Flowers MED, Storer B, Carpenter P, et al. Treatment change as a predictor of outcome among patients with classic chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2008;14:1380–4.
36. Akpek G, Zahurak M, Piantadosi S, et al. Development of a prognostic model for grading chronic graft-versus-host disease. Blood. 2001;97:1219–26.
37. Vigorito A, Campregher P, Storer B, et al. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood. 2009;114:702–8.

Biol Blood Marrow Transplant 19 (2013) 1690–1694

Comparison of Intravenous with Oral Busulfan in Allogeneic Hematopoietic Stem Cell Transplantation with Myeloablative Conditioning Regimens for Pediatric Acute Leukemia

Motohiro Kato [1,2,3,*], Yoshiyuki Takahashi [4], Daisuke Tomizawa [5], Yasuhiro Okamoto [6], Jiro Inagaki [7], Katsuyoshi Koh [3], Atsushi Ogawa [8], Keiko Okada [9], Yuko Cho [10], Junko Takita [1,2], Hiroaki Goto [11], Hisashi Sakamaki [12], Hiromasa Yabe [13], Keisei Kawa [14], Ritsuro Suzuki [15], Kazuko Kudo [16], Koji Kato [17]

[1] *Department of Cell Therapy and Transplantation Medicine, The University of Tokyo, Tokyo, Japan*
[2] *Department of Pediatrics, The University of Tokyo, Tokyo, Japan*
[3] *Department of Hematology/Oncology, Saitama Children's Medical Center, Saitama, Japan*
[4] *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[5] *Department of Pediatrics, Tokyo Medical and Dental University, Tokyo, Japan*
[6] *Department of Pediatrics, Kagoshima University, Kagoshima, Japan*
[7] *Department of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan*
[8] *Department of Pediatrics, Niigata Cancer Center Hospital, Niigata, Japan*
[9] *Department of Pediatric Hematology/Oncology, Osaka City General Hospital, Osaka, Japan*
[10] *Department of Pediatrics, Hokkaido University Hospital, Sappro, Japan*
[11] *Division of Hemato-oncology/Regenerative Medicine, Kanagawa Children's Medical Center, Yokohama, Japan*
[12] *Department of Hematology, Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, Tokyo, Japan*
[13] *Department of Cell Transplantation and Regenerative Medicine, Tokai University, Isehara, Japan*
[14] *Japanese Red Cross Kinki Block Blood Center, Osaka, Japan*
[15] *Department of HSCT Data Management and Biostatistics, Nagoya University, Nagoya, Japan*
[16] *Division of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, Japan*
[17] *Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan*

Article history:
Received 23 July 2013
Accepted 17 September 2013

Key Words:
Acute leukemia
Children
Busulfan

A B S T R A C T

Recent reports revealed that intravenous (iv) busulfan (BU) may not only reduce early nonrelapse mortality (NRM) but also improve overall survival (OS) probability in adults. Therefore, we retrospectively compared outcomes for 460 children with acute leukemia who underwent hematopoietic stem cell transplantation with either iv-BU (n = 198) or oral busulfan (oral-BU) (n = 262) myeloablative conditioning. OS at 3 years was 53.4% ± 3.7% with iv-BU and 55.1% ± 3.1% with oral-BU; the difference was not statistically significant ($P = .77$). OS at 3 years in 241 acute lymphoblastic leukemia and 219 acute myeloid leukemia patients was 56.4% ± 5.5% with iv-BU and 54.6% ± 4.1 with oral-BU ($P = .51$) and 51.0% ± 5.0% with iv-BU and 55.8% ± 4.8% with oral-BU ($P = .83$), respectively. Cumulative incidence of relapse at 3 years with iv-BU was similar to that with oral-BU (39.0% ± 3.6% and 36.4% ± 3.1%, respectively; $P = .67$). Cumulative incidence of NRM at 3 years was 16.6% ± 2.7% with iv-BU and 18.3% ± 2.5% with oral-BU ($P = .51$). Furthermore, multivariate analysis showed no significant survival advantage with iv-BU. In conclusion, iv-BU failed to show a significant survival advantage in children with acute leukemia.

INTRODUCTION

Busulfan (BU) is an alkylating agent that plays an important role in myeloablative preconditioning regimens in hematopoietic stem cell transplantation (HSCT) for patients with malignant diseases [1]. Although it has a potent antileukemic effect and excellent central nervous system penetration, wide inter- and intrapatient variability in absorption and metabolism has been observed with oral BU (oral-BU) [2]. However, its therapeutic potential has been compromised with unpredictable adverse events because overdosing leads to severe toxicity, and underdosing can potentially cause a relapse [3,4] or graft failure [5].

In children, BU is an important substitute for total body irradiation (TBI) [6], which is often associated with a higher incidence of late complications [7]; however, the range of heterogeneity in bioavailability with oral-BU is problematic. Blood concentrations and clearance may vary up to 6-fold among children receiving oral-BU [8,9], and age-dependent BU metabolism results in further complications. Therefore, therapeutic drug monitoring (TDM) of oral-BU has been considered an essential practice in children undergoing stem cell transplantation [10,11].

Intravenous BU (iv-BU) has recently replaced oral-BU because iv-BU avoids oral-BU's variable bioavailability. Furthermore, iv-BU showed less hepatic toxicity by avoiding the hepatic first-pass effect of oral-BU [12]. Previous reports revealed that the use of iv-BU reduced early

Financial disclosure: See Acknowledgments on page 1693.

* Correspondence and reprint requests: Motohiro Kato, MD, PhD, Department of Pediatrics, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.
E-mail address: katom-tky@umin.ac.jp (M. Kato).

1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.09.012

Table 1
Patients' Characteristics

Characteristics	Total, n	iv-BU, n (%)	Oral-BU, n (%)	*P* Value
All patients	460	198	262	
Age at HSCT				.23
<1 yr	83	34	49	
1 to 10 yrs	278	128	150	
>10 yrs	99	36	63	
Gender				.19
Male	237	95	142	
Female	223	103	120	
Year of HSCT				<.001
2000 to 2003	159	4	155	
2004 to 2007	155	52	103	
2008 to 2010	146	142	4	
Underlying disease				.01
ALL, total	241	90	151	
B lineage	198	76	122	
T lineage	22	9	13	
Not determined	21	5	16	
AML, total	219	108	111	
M0	7	4	3	
M1	31	11	20	
M2	37	18	19	
M3	3	2	1	
M4	31	12	19	
M5	46	24	22	
M6	6	3	3	
M7	48	28	20	
Not determined	7	5	2	
Disease status				.29
CR1	205	85	120	
CR2	74	28	46	
>CR2 and non-CR	179	85	94	
Unknown	2	0	2	
Prior HSCT				
No	343	147	196	
Yes	117	51	66	
Donor type				<.001
Related donor	189	71	118	
BM	131	54	77	
PB	58	17	41	
Unrelated donor BM	67	43	24	
Cord blood	199	83	116	
Unknown	5	1	4	
Usage in preconditioning regimens				
CY	219	87	132	.19
VP16	150	59	91	.27
L-PAM	226	101	125	.51

ALL indicates acute myeloid leukemia; AML, acute myeloid leukemia; iv-BU, intravenous busulfan; oral-BU, oral busulfan; CR, complete remission; HSCT, hematopoietic stem cell transplantation; BM, bone marrow; PB, peripheral blood; CY, cyclophosphamide; VP16, etoposide; L-PAM, melphalan.

complications, including hepatic sinusoidal obstruction syndrome (SOS) [13,14], and decreased early nonrelapse mortality (NRM) [13-15]. Some reports demonstrated that iv-BU may provide better overall survival (OS) in adults with malignant diseases [14-17].

Although several reports have been published on children with iv-BU [18-20], the number of patients included was small, and the reports mainly focused on acute toxicity or early clinical outcome because of a short follow-up period. Therefore, the role of iv-BU in HSCT for children with acute leukemia is yet to be determined.

In this study, to compare the clinical outcome of HSCT with iv-BU and oral-BU, we performed a retrospective analysis in 460 children who underwent myeloablative conditioning regimens including BU after allogeneic transplantation for acute leukemia.

METHODS

This study was approved by the institutional ethics committee of Saitama Children's Medical Center. A total 460 patients were analyzed based on the data reported in the Japan Society for Hematopoietic Cell Transplantation registry [21] (Table 1). The patients were selected based on the following criteria: (1) patients diagnosed with either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML); (2) age 15 years or younger when receiving HSCT; (3) BU-based myeloablative (more than 8 mg/kg) preconditioning regimens; and (4) HSCT performed between 2000 and 2010.

The OS probability was calculated using Kaplan-Meier estimates. Cumulative incidence curves were used in competing risks settings to calculate the probability of engraftment, graft-versus-host disease, and NRM. Univariate analyses of OS were performed using the log-rank test, and Gray's test was used for group comparisons of cumulative incidences. Multivariate analysis was performed using the Cox proportional hazard regression model, and the variables considered were patient's age, underlying diseases (ALL or AML), disease status (low risk: first complete remission [CR] or second CR, or high risk: nonremission or later than second CR), prior HSCT, donor type, and form of BU. All statistical analyses were performed using R software 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). A 2-sided *P* value of less than .05 was considered to be statistically significant.

RESULTS

The patients' characteristics are listed in Table 1. The median age at HSCT was 4 years (range, 0 to 15 years). Of the 460 HSCT patients, 198 used iv-BU and 262 used oral-BU. The iv-BU replaced oral-BU in most of the cases after the iv-BU approval in Japan in 2006 (Table 1). The median follow-up period was 1828 days (range, 85 to 4619 days) after HSCT in all the surviving patients and 1185 days (range, 100 to 3759 days) after HSCT in the iv-BU patients.

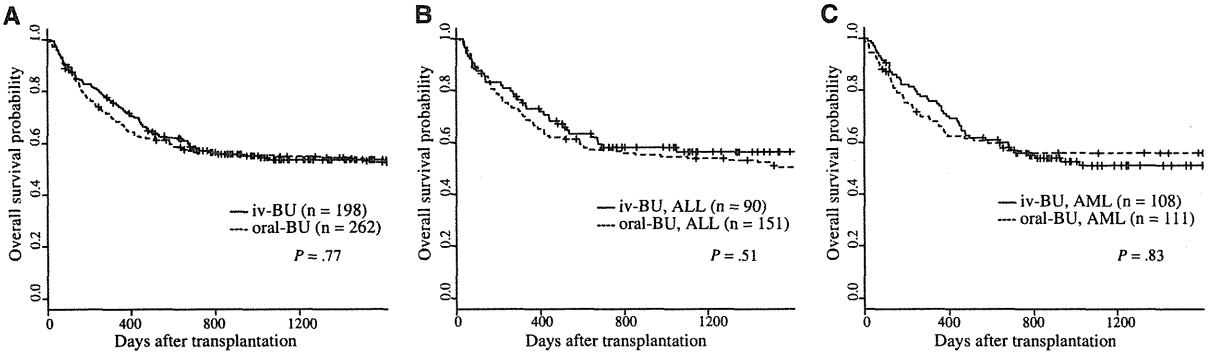

Figure 1. Overall survival probability of transplantation. Overall survival probability are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.

Table 2
Multivariate Analysis of the Risk Factors for Overall Mortality

Characteristics	No. of Patients	Overall Mortality	
		Hazard Ratio (95% CI)	P Value
Patient age at HSCT			
<1 yr	83	1	
1 to 9 yrs	278	1.18 (.76 to 1.86)	.45
>10 yrs	99	1.39 (.83 to 2.33)	.21
Underlying disease			
ALL	241	1	
AML	219	1.00 (.75 to 1.34)	.98
Disease status			
Low risk (CR1 and CR2)	279	1	
High risk (>CR2 and nonCR)	179	3.92 (2.86 to 5.39)	<.0001
Prior HSCT			
No	343	1	
Yes	117	1.47 (1.06 to 2.03)	.02
Donor type			
Related donor	189	1	
Unrelated donor (bone marrow)	67	1.09 (.72 to 1.64)	.68
Cord blood	199	1.22 (.90 to 1.66)	.19
Form of busulfan			
Oral	262	1	
Intravenous	198	.80 (.60 to 1.06)	.12

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; HSCT, hematopoietic stem cell transplantation.

The estimated OS probability and standard error at 3 years after HSCT was 54.6% ± 2.4%, whereas the cumulative incidence of relapse and NRM were 37.5% ± 2.3% and 17.6% ± 1.8%, respectively.

Although OS with iv-BU and oral-BU at day 100 after HSCT was 72.5% ± 3.2% and 66.9% ± 2.9%, respectively, OS at 3 years after HSCT was similar (iv-BU, 53.4% ± 3.7%; oral-BU, 55.1% ± 3.1%), and the log-rank test for OS did not show a statistically significant difference (P = .77) (Figure 1A). The result was concordant even when the analysis was limited to patients with ALL or AML. OS at 3 years for patients with ALL using iv-BU (n = 90) and oral-BU (n = 151) was 56.4% ± 5.5% and 54.6% ± 4.1%, respectively (P = .51) (Figure 1B). OS at 3 years for patients with AML using iv-BU (n = 108) and oral-BU (n = 111) was 51.0% ± 5.0% and 55.8% ± 4.8%, respectively (P = .83) (Figure 1C).

The similarity of OS was reproduced even with the limited cohort of 247 patients who received HSCT after the first CR or second CR without prior HSCT. OS at 3 years was 78.3% ± 4.2% for iv-BU patients (n = 98) and 78.7% ± 3.4% for oral-BU patients (n = 149) and the difference was not statistically significant (P = .66).

In addition, the hazard ratio of overall mortality between iv-BU and oral-BU was not statistically significant based on the multivariate analysis (Table 2).

The cumulative incidence curve of relapse after 3 years of iv-BU (39.0% ± 3.6%) was superimposed on the oral-BU curve (36.4% ± 3.1%) (Figure 2A). The difference did not show statistical significance after limiting the analyses to each disease. Relapse incidence at 3 years was 41.7% ± 5.5% with iv-BU and 39.1% ± 4.0% with oral-BU in the ALL cohort (P = .56) (Figure 2B), and 37.0% ± 4.8% with iv-BU and 33.6% ± 4.7% with oral-BU in the AML cohort (P = .70) (Figure 2C).

The cumulative incidence of NRM at 100 days after HSCT was 6.8% ± 1.8% with iv-BU and 8.3% ± 1.7% with oral-BU, and NRM at 3 years after HSCT was 16.6% ± 2.7% with iv-BU and 18.3% ± 2.5% with oral-BU (P = .51) (Figure 3A). The SOS occurrence was evaluable in 173 patients. Twenty-seven (30.3%) of 89 patients using iv-BU and 23 (27.4%) of 84 patients using oral-BU had SOS (P = .74). One patient using iv-BU and 4 patients using oral-BU succumbed to SOS. No significant difference in the causes of death was noted between the iv-BU and oral-BU groups.

The iv-BU group showed a tendency toward higher engraftment probability at day 60 (96.0% ± 1.5%) compared with the oral-BU group (89.3% ± 2.0%), but the difference was not statistically significant (P = .22) (Figure 3B). The cumulative incidence of acute graft-versus-host disease (grade II to IV) at day 100 was similar (iv-BU, 37.7% ± 3.5%; oral-BU, 35.4% ± 3.0%; P = .98).

DISCUSSION

Busulfan is widely used as an alternative myeloablative agent to TBI. Although previous randomized studies and a meta-analysis comparing BU with TBI revealed that TBI-based regimens were at least as good for survival and disease-free survival [22], it should be noted that majority of BU in these studies was oral-BU, and recent reports showed that targeted BU with TDM or iv-BU could improve HSCT outcome [10,11,14-17,23]. However, this study included the largest number of children to date and demonstrated that the advantage of iv-BU on survival probability was not significant. The result was reproduced in subgroups, such as patients with ALL, patients with AML, and patients who received iv-BU at first or second CR without prior HSCT.

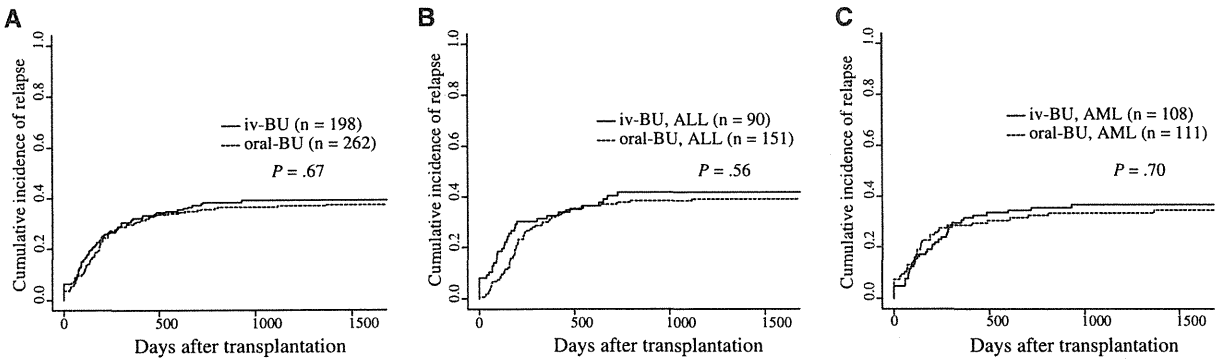

Figure 2. Cumulative incidences of relapse. Cumulative incidences of relapse are shown according to busulfan form, (A) in all patients, (B) in patients with ALL, and (C) in patients with AML.

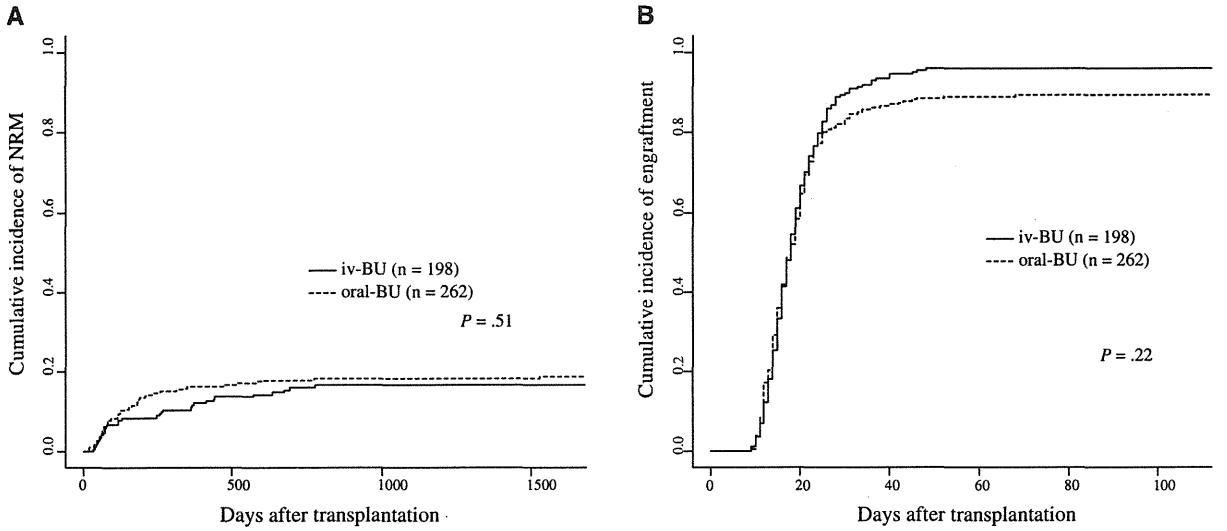

Figure 3. Cumulative incidences of nonrelapse mortality and engraftment. Cumulative incidences of (A) nonrelapse mortality and (B) engraftment are shown.

Moreover, multivariate analysis failed to show any survival benefit with iv-BU.

Our results regarding the iv-BU usage were discordant with the previous studies in adults. Although the reason is unclear, a possible explanation could be that the optimal dosing was already achieved in most patients, even those on oral-BU. In children, TDM of oral-BU has been considered an essential practice [10,11]. Therefore, the administration dose in oral-BU patients was usually determined based on the results of the test dose administration result and it was also adjusted according to TDM result.

Dosing schedule based on body weight using iv-BU provided adequate therapeutic targeting in children [24]. In our study, iv-BU failed to show superior outcomes compared with oral-BU, but it could provide a comparable survival outcome with a reduced requirement for TDM.

Concordant with previous studies, short-term NRM and OS in our study seemed to be superior in the iv-BU group compared with the oral-BU group, although it was caused by improvement of support therapy. Therefore, iv-BU may be advantageous for patients with high risk for treatment-related mortality, such as poor performance status, uncontrolled infectious diseases, or organ dysfunction.

This retrospective study, using the registry data, has some limitations. For example, the selection of iv-BU or oral-BU was strongly associated with the transplantation period, which may have introduced bias. Further prospective studies are required to establish an optimal allogeneic HSCT treatment strategy for children with acute leukemia.

In conclusion, our study provides valuable information on the role of iv-BU in myeloablative HSCT for pediatric acute leukemia. In children, iv-BU failed to improve the survival outcome of acute leukemia.

ACKNOWLEDGMENTS

The authors thank all the staff of the hospitals and centers, who have provided precise data via a registry of the Japan Society for Hematopoietic Cell Transplantation. A script kindly provided by Dr. Yoshinobu Kanda, Saitama Medical Center, Jichi Medical University, was used for data manipulation.

Authorship statement: M.K, Y.T, K.Kudo and K.Kato designed the research. J.I, K.Koh, A.O, K.O, H.S, H.Y, K.Kawa, R.S and K.Kato collected the data. M.K analyzed the data, and M.K, Y.T, D.T, Y.O, K.Kudo and K.Kato wrote the manuscript. All authors discussed the results and commented on the manuscript.

Conflict of interest statement: There are no conflicts of interest to report.

Financial disclosure: The authors have nothing to disclose.

REFERENCES

1. Santos GW, Tutschka PJ, Brookmeyer R, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *N Engl J Med.* 1983;309:1347-1353.
2. Hassan M, Ljungman P, Bolme P, et al. Busulfan bioavailability. *Blood.* 1994;84:2144-2150.
3. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood.* 1997;89:3055-3060.
4. Bolinger AM, Zangwill AB, Slattery JT, et al. An evaluation of engraftment, toxicity and busulfan concentration in children receiving bone marrow transplantation for leukemia or genetic disease. *Bone Marrow Transplant.* 2000;25:925-930.
5. Slattery JT, Sanders JE, Buckner CD, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant.* 1995;16:31-42.
6. Strahm B, Nollke P, Zecca M, et al. Hematopoietic stem cell transplantation for advanced myelodysplastic syndrome in children: results of the EWOG-MDS 98 study. *Leukemia.* 2011;25:455-462.
7. Michel G, Gluckman E, Esperou-Bourdeau H, et al. Allogeneic bone marrow transplantation for children with acute myeloblastic leukemia in first complete remission: impact of conditioning regimen without total-body irradiation—a report from the Societe Francaise de Greffe de Moelle. *J Clin Oncol.* 1994;12:1217-1222.
8. Schiltmeyer B, Klingebiel T, Schwab M, et al. Population pharmacokinetics of oral busulfan in children. *Cancer Chemother Pharmacol.* 2003; 52:209-216.
9. Tran HT, Madden T, Petropoulos D, et al. Individualizing high-dose oral busulfan: prospective dose adjustment in a pediatric population undergoing allogeneic stem cell transplantation for advanced hematologic malignancies. *Bone Marrow Transplant.* 2000;26:463-470.
10. Bleyzac N, Souillet G, Magron P, et al. Improved clinical outcome of paediatric bone marrow recipients using a test dose and Bayesian pharmacokinetic individualization of busulfan dosage regimens. *Bone Marrow Transplant.* 2001;28:743-751.
11. Bolinger AM, Zangwill AB, Slattery JT, et al. Target dose adjustment of busulfan in pediatric patients undergoing bone marrow transplantation. *Bone Marrow Transplant.* 2001;28:1013-1018.
12. Andersson BS, Madden T, Tran HT, et al. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and

cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant.* 2000;6:548-554.
13. Kim SE, Lee JH, Choi SJ, et al. Morbidity and non-relapse mortality after allogeneic bone marrow transplantation in adult leukemia patients conditioned with busulfan plus cyclophosphamide: a retrospective comparison of oral versus intravenous busulfan. *Haematologica.* 2005; 90:285-286.
14. Kashyap A, Wingard J, Cagnoni P, et al. Intravenous versus oral busulfan as part of a busulfan/cyclophosphamide preparative regimen for allogeneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortality, and overall 100-day mortality. *Biol Blood Marrow Transplant.* 2002;8:493-500.
15. Aggarwal C, Gupta S, Vaughan WP, et al. Improved outcomes in intermediate- and high-risk aggressive non-Hodgkin lymphoma after autologous hematopoietic stem cell transplantation substituting intravenous for oral busulfan in a busulfan, cyclophosphamide, and etoposide preparative regimen. *Biol Blood Marrow Transplant.* 2006;12: 770-777.
16. Sobecks RM, Rybicki L, Yurch M, et al. Intravenous compared with oral busulfan as preparation for allogeneic hematopoietic progenitor cell transplantation for AML and MDS. *Bone Marrow Transplant.* 2012;47: 633-638.
17. Dean RM, Pohlman B, Sweetenham JW, et al. Superior survival after replacing oral with intravenous busulfan in autologous stem cell transplantation for non-Hodgkin lymphoma with busulfan, cyclophosphamide and etoposide. *Br J Haematol.* 2010;148:226-234.
18. Schechter T, Finkelstein Y, Doyle J, et al. Pharmacokinetic disposition and clinical outcomes in infants and children receiving intravenous busulfan for allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2007;13:307-314.
19. Zwaveling J, Bredius RG, Cremers SC, et al. Intravenous busulfan in children prior to stem cell transplantation: study of pharmacokinetics in association with early clinical outcome and toxicity. *Bone Marrow Transplant.* 2005;35:17-23.
20. Bartelink IH, Bredius RG, Ververs TT, et al. Once-daily intravenous busulfan with therapeutic drug monitoring compared to conventional oral busulfan improves survival and engraftment in children undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2008;14:88-98.
21. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol.* 2007;86:269-274.
22. Hartman AR, Williams SF, Dillon JJ. Survival, disease-free survival and adverse effects of conditioning for allogeneic bone marrow transplantation with busulfan/cyclophosphamide vs total body irradiation: a meta-analysis. *Bone Marrow Transplant.* 1998;22:439-443.
23. Deeg HJ, Storer B, Slattery JT, et al. Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood.* 2002;100:1201-1207.
24. Michel G, Valteau-Couanet D, Gentet JC, et al. Weight-based strategy of dose administration in children using intravenous busulfan: clinical and pharmacokinetic results. *Pediatr Blood Cancer.* 2012;58:90-97.

To the editor:

Rabbit antithymocyte globulin and cyclosporine as first-line therapy for children with acquired aplastic anemia

Horse antithymocyte globulin (hATG) and cyclosporine have been used as standard therapy for children with acquired aplastic anemia (AA) for whom an HLA-matched family donor is unavailable. However, in 2009, hATG (lymphoglobulin; Genzyme) was withdrawn and replaced by rabbit ATG (rATG; thymoglobulin; Genzyme) in Japan. Many other countries in Europe and Asia are facing the same situation.[1] Marsh et al recently reported outcomes for 35 adult patients with AA who were treated with rATG and cyclosporine as a first-line therapy.[2] Although the hematologic response rate was 40% at 6 months, several patients subsequently achieved late responses. The best response rate was 60% compared with 67% in a matched-pair control group of 105 patients treated with hATG. The overall and transplantation-free survival rates appeared to be significantly inferior with rATG compared with hATG at 68% versus 86% ($P = .009$) and 52% versus 76% ($P = .002$), respectively. These results are comparable to those from a prospective randomized study reported by Scheinberg et al comparing hATG and rATG.[3] Both studies showed the superiority of hATG over rATG.[2,3]

We recently analyzed outcomes for 40 Japanese children (median age, 9 years; range, 1-15) with AA treated using rATG and cyclosporine. The median interval from diagnosis to treatment was 22 days (range, 1-203). The numbers of patients with very severe, severe, and nonsevere disease were 14, 10, and 16, respectively. The ATG dose was 3.5 mg/kg/day for 5 days. The median follow-up time for all patients was 22 months (range, 6-38). At 3 months, no patients had achieved a complete response (CR) and partial response (PR) was seen in only 8 patients (20.0%). At 6 months, the numbers of patients with CR and PR were 2 (5.0%) and 17 (42.5%), respectively. After 6 months, 5 patients with PR at 6 months had achieved CR and 4 patients with no response at 6 months had achieved PR, offering a total best response rate of 57.5%. Two patients relapsed at 16 and 19 months without receiving any second-line treatments. Two patients with no response received a second course of rATG at 13 and 17 months, but neither responded. Sixteen patients underwent hematopoietic stem cell transplantation (HSCT) from alternative donors (HLA-matched unrelated donors, n = 13; HLA-mismatched family donors, n = 3). Two deaths occurred after rATG therapy, but no patients died after HSCT. Causes of death were intracranial hemorrhage at 6 months and acute respiratory distress syndrome at 17 months. The overall 2-year survival rate was 93.8% and the 2-year transplantation-free survival rate was 50.3% (Figure 1).

In our previous prospective studies with hATG, the response rates after 6 months were 68% and 70%, respectively, with no increases in response rates observed after 6 months.[4,5] Our results support the notion that rATG is inferior to hATG for the treatment of AA in children. First-line HSCT from an alternative donor may be justified, considering the excellent outcomes in children who received salvage therapies using alternative donor HSCT.

Yoshiyuki Takahashi
Department of Pediatrics, Nagoya Graduate School of Medicine, Nagoya, Japan

Hideki Muramatsu
Department of Pediatrics, Nagoya Graduate School of Medicine, Nagoya, Japan

Naoki Sakata
Department of Pediatrics, Kinki University School of Medicine, Osaka, Japan

Nobuyuki Hyakuna
Center of Bone Marrow Transplantation, Ryukyu University Hospital, Okinawa, Japan

Kazuko Hamamoto
Department of Pediatrics, Hiroshima Red Cross Hospital, Hiroshima, Japan

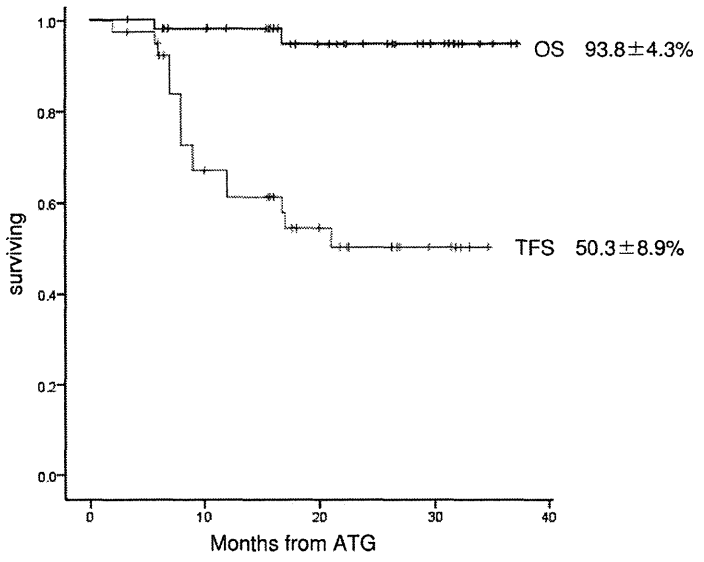

Figure 1. Kaplan-Meier estimates of overall survival (OS) and transplantation-free survival (TFS) in 40 Japanese children with AA. Survival was investigated using Kaplan-Meier methods. OS for all patients with AA after rATG and cyclosporine as first-line therapy included patients who later received HSCT for nonresponse to rATG. In the analysis of TFS for all patients treated with rATG and CSA, transplantation was considered an event.

Ryoji Kobayashi
Department of Pediatrics, Sapporo Hokuyu Hospital, Sapporo, Japan

Etsuro Ito
Department of Pediatrics, Hirosaki University School of Medicine, Hirosaki, Japan

Hiroshi Yagasaki
Department of Pediatrics, School of Medicine, Nihon University, Tokyo, Japan

Akira Ohara
Division of Blood Transfusion, Toho University Omori Hospital, Tokyo, Japan

Akira Kikuchi
Department of Pediatrics, Teikyo University School of Medicine, Tokyo, Japan

Akira Morimoto
Department of Pediatrics, Jichi Medical University School of Medicine, Tochigi, Japan

Hiromasa Yabe
Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan

Kazuko Kudo
Division of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, Japan

Ken-Ichiro Watanabe
Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Shouichi Ohga
Department of Perinatal and Pediatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Seiji Kojima
Department of Pediatrics, Nagoya Graduate School of Medicine, Nagoya, Japan

on behalf of the Japan Childhood Aplastic Anemia Study Group

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Dr Seiji Kojima, Nagoya Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya, Ai, Japan 466-8550; e-mail: kojimas@med.nagoya-u.ac.jp.

References

1. Dufour C, Bacigalupo A, Oneto R, et al. Rabbit ATG for aplastic anaemia treatment: a backward step? *Lancet.* 2011;378(9806):1831-1833.
2. Marsh JC, Bacigalupo A, Schrezenmeier H, et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. *Blood.* 2012;119(23):5391-5396.
3. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med.* 2011;365(5):430-438.
4. Kojima S, Hibi S, Kosaka Y, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. *Blood.* 2000;96(6):2049-2054.
5. Kosaka Y, Yagasaki H, Sano K, et al. Prospective multicenter trial comparing repeated immunosuppressive therapy with stem-cell transplantation from an alternative donor as second-line treatment for children with severe and very severe aplastic anemia. *Blood.* 2008;111(3):1054-1059.

To the editor:

Peripheral blood stem cells versus bone marrow in pediatric unrelated donor stem cell transplantation

The relative benefits and risks of peripheral blood stem cells (PBSCs) versus bone marrow (BM) for allogeneic hematopoietic stem cell transplantation (SCT) are still a matter of highly controversial debates.[1-3] The first randomized study comparing the 2 stem cell sources in unrelated donor SCT recently documented comparable overall and event-free survival, but indicated a higher risk for chronic graft-versus-host disease (GVHD) with PBSCs.[4] Only a few pediatric patients were included in this study even though the long-term sequelae of chronic GVHD are of particular concern in this patient group.

We retrospectively compared the long-term outcome of contemporaneous unrelated donor SCT in 220 children transplanted with BM (n = 102) or PBSCs (n = 118) for hematologic malignancies and reported to the German/Austrian pediatric registry for SCT. All patients had received myeloablative conditioning followed by unmanipulated SCT from HLA-matched unrelated donors. The PBSC and BM groups were comparable with regard to patient and donor age, sex, cytomegalovirus (CMV) serostatus, disease status at transplantation, GVHD prophylaxis, growth factor use, and degree of HLA matching. The groups differed with regard to disease category with slightly more myelodysplastic syndrome patients ($P = .02$) and a higher CD34-cell dose ($P = .001$) in the PBSC group.

Neutrophil and platelet engraftment were achieved significantly faster after PBSC than BM transplantation (Figure 1A-B). In this entirely pediatric cohort, the incidence of clinically relevant grade II-IV acute GVHD (Figure 1C) did not differ. Most importantly, the incidence of chronic GVHD (PBSCs vs BM: 35% vs 33%, respectively; $P = .9$) and extensive chronic GVHD (Figure 1D) proved low and was virtually identical in the 2 groups. With a median follow-up time of 3 years, overall survival (PBSCs vs BM: 50% ± 5% vs 46% ± 6%, respectively; $P = .63$) and event-free survival (PBSCs vs BM: 45% ± 5% vs 44% ± 6%, respectively; $P = .59$) were comparable (Figure 1E-F). In multivariable analysis, taking into account all parameters with $P < .2$ in univariate analysis, the only significant independent risk factor for treatment failure was advanced disease status at the time of transplantation (relative risk = 2.4, 95% confidence interval, 1.5-3.8; $P = .001$). In contrast, stem cell source (PBSCs vs BM) had no effect (relative risk = 1.1, 95% confidence interval, 0.7-1.6; $P = .8$).

Our registry-based analysis provides evidence that in pediatric recipients of HLA-matched unrelated-donor transplantation with consistent antithymocyte globulin (ATG) use during conditioning, transplantation with PBSCs and BM results in comparable clinical outcomes without detectable differences in the risk of acute or, more importantly, chronic GVHD. Consistent with a recent study underscoring the role of ATG for the prevention of acute and chronic GVHD,[5] the use of ATG in 96% of our transplantation procedures compared with only 27% in the above-mentioned randomized study by Anasetti et al[4] might be one of the key factors responsible for the overall low and comparable incidence of

Int J Hematol (2012) 95:675–679
DOI 10.1007/s12185-012-1079-9

ORIGINAL ARTICLE

Excellent outcome of allogeneic bone marrow transplantation for Fanconi anemia using fludarabine-based reduced-intensity conditioning regimen

Akira Shimada · Yoshiyuki Takahashi · Hideki Muramatsu · Asahito Hama · Olfat Ismael · Atsushi Narita · Hiroshi Sakaguchi · Sayoko Doisaki · Nobuhiro Nishio · Makito Tanaka · Nao Yoshida · Kimikazu Matsumoto · Koji Kato · Nobuhiro Watanabe · Seiji Kojima

Received: 21 October 2011 / Revised: 5 April 2012 / Accepted: 5 April 2012 / Published online: 22 April 2012

Abstract Fanconi anemia (FA) is a disorder characterized by developmental anomalies, bone marrow failure and a predisposition to malignancy. It has recently been shown that hematopoietic stem cell transplantation using fludarabine (FLU)-based reduced-intensity conditioning is an efficient and quite safe therapeutic modality. We retrospectively analyzed the outcome of bone marrow transplantation (BMT) in eight patients with FA performed in two institutes between 2001 and 2011. There were seven females and one male with a median age at diagnosis = 4.5 years (range 2–12 years). The constitutional characteristics associated with FA, such as developmental anomalies, short stature and skin pigmentation, were absent in three of the patients. One patient showed myelodysplastic features at the time of BMT. All patients received BMT using FLU, cyclophosphamide (CY) and rabbit antithymocyte globulin (ATG) either from a related donor ($n = 4$) or an unrelated donor ($n = 4$). Acute graft-versus-host disease (GVHD) of grade I developed in one patient, while chronic GVHD was not observed in any patient. All patients are alive and achieved hematopoietic recovery at a median follow-up of 72 months (range 4–117 months). BMT using FLU/low-dose CY/ATG -based regimens regardless to the donor is a beneficial therapeutic approach for FA patients.

Keywords Fanconi anemia · Hematopoietic stem cell transplantation · Fludarabine

A. Shimada · Y. Takahashi · H. Muramatsu · A. Hama · O. Ismael · A. Narita · H. Sakaguchi · S. Doisaki · N. Nishio · M. Tanaka · S. Kojima (✉)
Department of Pediatrics,
Nagoya University Graduate School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan
e-mail: kojimas@med.nagoya-u.ac.jp

A. Shimada
e-mail: ashimada@med.nagoya-u.ac.jp

N. Yoshida · K. Matsumoto · K. Kato
Department of Pediatric Hematology/Oncology,
Nagoya First Red Cross Hospital, 3-35 Dougecho,
Nakamuraku, Nagoya 453-8511, Japan

N. Watanabe
Department of Pediatrics, Cyukyo Hospital,
1-1-10 Sanjo, Minamiku, Nagoya 457-0866, Japan

Introduction

Fanconi anemia (FA) is a complex disorder characterized by developmental anomalies, early onset progressive bone marrow failure (BMF) and a tendency to develop hematological and non-hematological malignancies. The risk of FA patients to develop BMF and malignancies increases with progression of age, and the cumulative incidence by age of 40 years was 90 % for BMF and 30 % for hematologic and nonhematologic neoplasms [1–4]. Short stature and abnormal skin pigmentation are particularly common features found in patients with FA and a wide variety of congenital malformations has been described in 60–75 % of FA patients [3–5]. The incidence of FA in Japan was approximately 5–10 new cases diagnosed annually and hematological abnormalities usually manifested early in childhood at a median age of 7 years (range 0–31 years). Owing to the complexity of the disease and multisystem involvement, FA has a high mortality rate with a median age of death of 30 years [3–5]. Therefore, the study of FA holds a great promise for elucidation of the heterogeneity of this disorder in the future. Hematopoietic stem cell transplantation (HSCT) is the only curative therapy known so far for correcting the hematological manifestations in

Table 1 Clinical characteristics of 8 Fanconi anemia patients

No.	Male/ female	Disease status at HSCT	Age at diagnosis (year)	Age at HSCT (year)	Karyotype	Chromosomal fragility test (gap and break/100 cells)	Short stature (<−1SD)	Skin pigmentation	Other anomalies
1	F	SAA	7	8	46,XX	114	No	No	No
2	F	SAA	3	5	46,XX	514	−2SD	Yes	Shortness of first finger
3	F	SAA	8	8	46,XX	193	No	No	No
4	F	SAA	12	13	46,XX	299	−1SD	Yes	No
5	M	SAA	2	7	46,XY	152	−3SD	No	Hyperdactylia
6	F	MDS (RAEB)	3	6	Other[a]	148	−1.5SD	No	Hyperdactylia
7	F	SAA	6	11	46,XX	159	No	No	No
8	F	SAA	3	5	46,XX	169	−2SD	Yes	Radius defect, esophageal atresia

SAA severe aplastic anemia, *MDS* myelodysplastic syndrome, *HSCT* hematopoietic stem cell transplantation, *RAEB* refractory anemia with excess blasts

[a] others; 46,XX,add(3)(q26),der(7)add(7)(p22)add(7)(q11)add(8)(q22)

FA patients. Initially, potential problems in designing HSCT conditioning regimens for patients with FA appeared due to the acquired hypersensitivity to DNA cross-linking or oxidative agents such as alkylating agents or ionizing radiation [6], but recently a significant progress has been achieved by using FLU-based reduced-intensity conditioning regimens that markedly improved the efficiency and safety of this procedure [7, 8]. Specifically, FLU-based reduced-intensity conditioning allogeneic HSCT resulted in reduction of regimen-related toxicity (RRT), superior engraftment and less graft-versus-host disease (GVHD), which in turn led to improvement of patients' survival. In this study, we aimed to investigate the effectiveness and safety profile of bone marrow transplantation (BMT) using FLU-based reduced-intensity conditioning regimens in eight patients with FA, who were transplanted from either a related donor (3 HLA-genetically matched and 1 HLA-A locus mismatched) or an unrelated donor (2 HLA-matched and 2 HLA-DRB1 mismatched).

Patients and methods

We retrospectively analyzed eight patients, who were diagnosed as having FA and received BMT at Nagoya University and Nagoya First Red Cross Hospital between 2001 and 2011. There were seven females and one male with a median age at diagnosis of 4.5 years (range 2–12 years) (Table 1). Clinical features suggestive of FA including low birth weight, short stature, hyperpigmented skin, radial abnormality and duplicated thumbs were defined in five out of eight patients, while three patients were asymptomatic. Diagnosis of FA was confirmed in all patients by a reliable cellular marker for FA cells and all of them showed a high incidence of chromosomal breaks and gaps, which indicated chromosomal instability by adding mitomycin C at a final concentration of 0.5 μM (Table 1). Seven patients suffered from severe aplastic anemia and one patient evolved to refractory anemia with excess of blasts (RAEB) with the emergence of cytogenetic abnormalities in the form of add(3)(q26) at the time of HSCT. All patients underwent allogeneic BMT at a median age of 7.5 years (range 5–13 years), and they were transplanted from either a related donor (3 HLA-genetically matched and 1 HLA-A locus mismatched) or an unrelated donor (2 HLA- matched and 2 HLA-DRB1 mismatched). All patients received a preparative regimen including a combination of fludarabine (FLU 120–180 mg/m^2), cyclophosphamide (CY 40 mg/kg) and rabbit anti-thymocyte globulin (ATG, Thymoglobulin, Genzyme, 5–10 mg/kg). Patients transplanted from unrelated donor received a total body irradiation (TBI)/total lymphoid irradiation (TLI) of 4–4.5 Gy, and patients transplanted from HLA-A locus mismatched related donor received 2 Gy. As GVHD prophylaxis, BM recipients from related donor received cyclosporine A (CyA) plus short-term methotrexate (MTX), while BM recipients from unrelated donor received tacrolimus (FK506) plus short-term MTX. Details on the donors' characteristics, conditioning regimen and GVHD prophylaxis are listed in Table 2.

Table 2 Hematopoietic stem cell transplantation for 8 Fanconi anemia patients

No.	Performance status	Transfusion before SCT	Conditioning	Donor	Concordance of HLA-serological typing	Transfused cell number ($\times 10^8$/kg)	GVHD prophylaxis
1	0	MAP 2u, PC10u	FLU (120 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg)	Mother	6/6	3.9	CyA + short MTX
2	0	MAP 2u, PC10u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (6 mg/kg)	Sister	6/6	7.4	CyA + short MTX
3	0	MAP 4u, PC 20u	FLU (180 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (2 Gy)	Father	5/6 (mismatched A 1 locus)	3.0	CyA + short MTX
4	0	MAP 4u, PC 20u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (5 mg/kg)	Brother	6/6	2.2	CyA + short MTX
5	1	MAP 21u, PC 120u	FLU(180 mg/m^2) + CY(40 mg/kg) + ATG(10 mg/kg) + TLI (4 Gy)	Unrelated	6/6	3.0	FK506 + short MTX
6	0	MAP 16u, PC100u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (4.5 Gy)	Unrelated	5/6 (mismatched DR 1 locus)	4.5	FK506 + short MTX
7	0	MAP 12u, PC120u	FLU (120 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TLI (4 Gy)	Unrelated	5/6 (mismatched DR 1 locus)	3.8	FK506 + short MTX
8	0	MAP 4u, PC 20u	FLU (150 mg/m^2) + CY (40 mg/kg) + ATG (10 mg/kg) + TBI (4.5 Gy)	Unrelated	6/6	4.6	FK506 + short MTX

SCT stem cell transplantation, *FLU* fludarabine, *CY* cyclophosphamide, *ATG* anti-thymocyte globulin (rabbit ATG, Thymoglobulin), *TBI* total body irradiation, *TLI* total lymphoid irradiation, *CyA + short MTX* cyclosporine plus short-term methotrexate

Results

The median transfused nucleated cell number was 3.8 × 10^8/kg (range 2.2 to 7.4 × 10^8/kg) and all patients achieved sustained engraftment; the median time to neutrophil (>500), platelet (>50,000/ml) and reticulocyte (>10‰) recovery was 15.5, 20.5 and 21.5 days, respectively. Among eight patients enrolled in this study with a median survival period of 72 months (range 4–117 months), acute GVHD grade I was detected in one patient, whereas chronic GVHD was not found in any patient. Two patients experienced hepatic dysfunction and one patient had gastric hemorrhage as regimen-related toxicities (grade 1 according to the National Cancer Institution-Common Toxicity Criteria, NCI-CTC Version 4.0). Three patients exhibited febrile neutropenia and one of them showed disseminated fungal infection (grade 4 according to NCI-CTC) complicated by development of a renal abscess that showed complete remission after amphotericin B treatment. Cytomegalovirus (CMV)-polymerase chain reaction (PCR) and CMV-pp65 antigen detection were performed on a weekly basis for identification of CMV infection. We found four out of eight patients showed CMV reactivation without clinical symptom and they were treated with ganciclovir and foscavir. One patient suffered from hemorrhagic cystitis and lymphoproliferative disorder (LPD) due to BK virus and Epstein–Barr virus (EBV) reactivation, respectively. This patient was completely cured from the EBV–LPD after successful treatment with rituximab. Otherwise, no patients developed veno-occlusive disease (VOD) or thrombotic microangiopathy (TMA) (Table 3). Over the total length of the follow-up period, no patients showed secondary bone marrow failure and/or malignancies with a median follow-up of 72 months (range 4–117 months).

Discussion

Through a retrospective analysis of the medical records of eight pediatric patients with FA, who received FLU-based reduced-intensity conditioning allogeneic HSCT to evaluate their outcome, we found that all patients achieved favorable outcome using this procedure and the type of the donor did not significantly influence the clinical outcome. In previous studies, it has been proved that the use of alkylating agents and radiation therapy for FA patients was harmful. The impact of lower irradiation dose on immune recovery and risk of malignancy remains a matter of debate and a longer follow-up period is needed. However, it was reported that TBI 300 cGy was the lowest possible irradiation dose in the context of FLU/CY-based regimen; other

Table 3 Results of hematopoietic stem cell transplantation for 8 Fanconi anemia patients

No.	Days of engraftment	Acute GVHD	Chronic GVHD	RRT	VOD	TMA	CMV Ag positivity and treatment	Other virus related disease	FN	Fungal infection	Overall survival (Mo)
1	19	0	0	No	No	No	Day 35, GCV	No	No	No	114
2	15	I	0	No	No	No	Day 13, GCV + FCV	No	Yes	No	41
3	19	0	0	Liver dysfunction (grade 1)	No	No	Day 30, GCV	No	Yes	No	17
4	14	0	0	Gastric hemorrhage (grade 1)	No	No	No	No	No	No	4
5	17	0	0	No	No	No	Day 48, GCV	No	No	No	117
6	19	0	0	No	No	No	No	No	No	Disseminated fungal infection, renal abscess (grade 4)	99
7	15	0	0	Diarrhea (grade 1), liver dysfunction (grade 1)	No	No	No	Hemorrhagic cystitis due to BKV (grade 2) and LPD due to EBV	Yes	No	72
8	19	0	0	No	No	No	No	No	No	No	26

RRT grade was determined by National Cancer Institute-Common Toxicity Criteria (NCI-CTC Ver 4.0)

GVHD graft-versus-host disease, *RRT* regimen-related toxicity, *VOD* veno-occlusive disease, *TMA* thrombotic microangiopathy, *CMV Ag* AMV antigenemia (pp65), *GCV* ganciclovir, *FCV* foscavir, *BKV* BK virus, *EBV*, Epstein–Barr virus, *FN* febrile neutropenia

successful trials of eliminating irradiation in the conditioning regimens even in recipients of BMT from unrelated donor have been achieved [8–11]. Subsequently, alternative regimens have been developed to reduce the potential risk of irradiation and GVHD using a non-irradiation based preparative therapy including a low-dose CY, FLU and ATG [6–8, 10]. Here, we showed that BMT using FLU-based reduced-intensity conditioning regimens led to improvement of the outcome for FA patients regardless of the donor type. In line with our findings, other investigators reported that HSCT using FLU-containing regimens was associated with a better outcome even for recipients of allograft from unrelated donor with stable engraftment and minimal toxicity, whereas adding ATG to the conditioning regimen contributed to decreasing the incidence of GVHD [7, 8, 11, 12]. On the other hand, it was reported that bone marrow recipients from unrelated donor showed less successful transfusion rate with high percentage of graft failure and RRT compared to bone marrow recipients from related donor [13]. In our cohort, FLU/ATG/low-dose CY-based conditioning regimen was tolerable and efficient for FA patients even for BM recipient from unrelated (HLA-1 locus mismatched) donor, but some patients developed virus reactivation such as CMV, BKV and EBV. To overcome virus reactivation that might occur as an adverse event following the use of ATG-containing regimen, it is better to decrease the ATG dose from 10 to 5 mg/kg. FA patients are more prone to cancer development such as squamous cell carcinomas with special predilection sites (esophagus, head and neck) [4, 14, 15]. FLU-containing regimens were employed in a limited number of FA patients, making it difficult to speculate on its implications on cancer progression. Three out of four patients received HSCT from HLA-matched related donor without irradiation and they showed successful and excellent outcomes. Therefore, it is important to consider the use of the conditioning regimen without irradiation for this group of patients. Several studies have demonstrated that TBI with 300–450 cGY is needed for consistent engraftment in recipients from unrelated donor [12, 16]. We would like to emphasize that reduction of irradiation dose in the conditioning regimens even for recipients of HSCT from unrelated donor is an important target that will improve the patient's quality of life by reducing late effects, particularly the risk of malignancy. Noteworthy, in our series three out of eight patients were asymptomatic. Although our findings were consistent with other investigators' results [3–5], early diagnosis and optimal timing of transplant in

asymptomatic FA patients is challenging and it is of utmost importance to confirm FA diagnosis in those patients who might be misdiagnosed with acquired aplastic anemia. In conclusion, the identification of asymptomatic FA patients requires careful consideration by testing for cross-linker hypersensitivity that provides a reliable cellular marker for FA diagnosis. HSCT using FLU/low-dose CY/ATG-based regimen is beneficial and could be a promising therapeutic approach for FA patients regardless of the donor type with favorable clinical outcome.

Acknowledgments This work was supported in part by grant for scientific research by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Conflict of interest The authors declare no conflict of interest.

References

1. Fanconi G. Familial constitutional panmyelocytopathy, Fanconi's anemia (F.A.). I. Clinical aspects. Semin Hematol. 1967;4: 233–40.
2. Alter BP. Fanconi's anemia and malignancies. Am J Hematol. 1996;53:99–110.
3. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, Auerbach AD. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003;101:1249–56.
4. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24: 101–22.
5. Auerbach AD. Fanconi anemia and its diagnosis. Mutat Res. 2009;668:4–10.
6. Yabe M, Yabe H, Hamanoue S, Inoue H, Matsumoto M, Koike T, Ishiguro H, Morimoto T, Arakawa S, Ohshima T, Masukawa A, Miyachi H, Yamashita T, Katob S. In vitro effect of fludarabine, cyclophosphamide, and cytosine arabinoside on chromosome breakage in Fanconi anemia patients: relevance to stem cell transplantation. Int J Hematol. 2007;85(4):354–61.
7. Yabe H, Inoue H, Matsumoto M, Hamanoue S, Koike T, Ishiguro H, Koike H, Suzuki K, Kato S, Kojima S, Tsuchida M, Mori T, Adachi S, Tsuji K, Koike K, Morimoto A, Sako M, Yabe M. Allogeneic haematopoietic cell transplantation from alternative donors with a conditioning regimen of low-dose irradiation, fludarabine and cyclophosphamide in Fanconi anaemia. Br J Haematol. 2006;134:208–12.
8. MacMillan ML, Wagner JE. Haematopoietic cell transplantation for Fanconi anaemia—when and how? Br J Haematol. 2010; 149:14–21.
9. Gluckman E, Berger R, Dutreix J. Bone marrow transplantation for Fanconi anemia. Semin Hematol. 1984;21:20–6.
10. Kapelushnik J, Or R, Slavin S, Nagler A. A fludarabine-based protocol for bone marrow transplantation in Fanconi's anemia. Bone Marrow Transplant. 1997;20:1109–10.
11. Dalle JH. HSCT for Fanconi anemia in children: factors that influence early and late results. Bone Marrow Transplant. 2008; 42:S51–3.
12. Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, Heller G, Wolden S, O'Reilly RJ, Boulad F. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. Br J Haematol. 2008;140:644–55.
13. Gluckman E, Auerbach AD, Horowitz MM, Sobocinski KA, Ash RC, Bortin MM, Butturini A, Camitta BM, Champlin RE, Friedrich W, Good RA, Gordon-Smith EC, Harris RE, Klein JP, Ortega JJ, Pasquini R, Ramsay NK, Speck B, Vowels MR, Zhang MJ, Gale RP. Bone marrow transplantation for Fanconi anemia. Blood. 1995;86:2856–62.
14. Rosenberg PS, Socié G, Alter BP, Gluckman E. Risk of head and neck squamous cell cancer and death in patients with Fanconi anemia who did and did not receive transplants. Blood. 2005;105: 67–73.
15. Masserot C, Peffault de Latour R, Rocha V, Leblanc T, Rigolet A, Pascal F, Janin A, Soulier J, Gluckman E, Socié G. Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. Cancer. 2008;113:3315–22.
16. MacMillan ML, Blazar BR, DeFor TE, Dusenbery K, Wagner JE. Thymic shielding (TS) in recipients of total body irradiation (TBI) and alternative donor hematopoietic stem cell transplant (AD-HSCT): reduced risk of opportunistic infection in patients with Fanconi anemia (FA). Blood. 2006;108 (abstract #3134).