も試行錯誤している。患者会などの協力を得て，今後グリーフケアについても取り組んでいきたい。

おわりに―医師の役割

悪性腫瘍の患児と家族の精神的サポートには多職種のかかわりが必須である。そのなかで医師の果たすべき役割は，全体を俯瞰して方向性を見定める先導役と，各職種が十分に機能できるように環境を整える調整役ではないかと考えている。

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Outcomes of Immunological Interventions for Mixed Chimerism Following Allogeneic Stem Cell Transplantation in Children With Juvenile Myelomonocytic Leukemia

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Background. For children with juvenile myelomonocytic leukemia (JMML) who undergo stem cell transplantation (SCT), the role of immunological interventions including withdrawal of immunosuppressive therapy (IST) and donor lymphocyte infusion (DLI) for treatment of disease recurrence remains uncertain. ***Procedure.*** We analyzed serial chimerism status following SCT and evaluated the efficacy of immunological interventions for the management of mixed chimerism (MC) in children with JMML. ***Results.*** Chimerism analysis was available in 26 SCT cases following the first and second SCT. MC was observed in 16 cases and withdrawal of IST was performed in 14 cases immediately after identification of MC. Donor lymphocyte infusion (DLI) was performed in five MC cases. Eight MC cases were observed at the time of neutrophil recovery. Following withdrawal of IST, three cases achieved complete chimerism (CC) while the proportion of autologous cells increased rapidly in the remaining five cases. Six MC cases were observed after achievement of hematological remission (HR) and responses to withdrawal of IST were observed in two cases. In the remaining four cases, despite withdrawal of IST, the proportion of autologous cells increased. Five cases received DLI but only one case responded. ***Conclusion.*** Although the benefits of immunological interventions for MC after SCT in JMML were limited, some patients did achieve HR as a result of these treatment modalities without a second SCT. Close monitoring of donor chimerism and early detection of MC is helpful in guiding treatment after SCT in children with JMML. Pediatr Blood Cancer 2013;60:116–120.

Key words: immunological intervention; JMML; mixed chimerism; stem cell transplantation

INTRODUCTION

Juvenile myelomonocytic leukemia (JMML) is a rare hematopoietic disorder of early childhood, accounting for about 2% of all pediatric hematological malignancies [1]. The median survival time without allogeneic stem cell transplantation (SCT) is approximately 1 year [2,3]. SCT is presently the only curative treatment and recent studies have shown that about 50% of the children with JMML could be cured by SCT [4–6]. In these studies, relapse rates of approximately 30–50% were reported and disease recurrence remains the major cause of treatment failure after SCT for JMML. Relapse occurs at a median of 2–6 months after SCT and generally within the first year following SCT [2–4].

It has been suggested that the graft-versus-leukemia (GVL) effect may play an important role in achieving a cure of JMML. For example, lower relapse rates after SCT were observed in patients who developed graft-versus-host disease (GVHD) [5,7,8]. Several case reports showed successful treatment of patients who achieved full donor chimerism by withdrawal of immunosuppressive therapy (IST) or donor lymphocyte infusion (DLI) following relapse after SCT [9–13]. Yoshimi et al. [14] reported the results of serial chimerism status and immunological intervention following SCT in 30 children with JMML. Increasing recipient chimerism was noted in 12 patients, of whom 10 relapsed and two achieved complete donor chimerism following withdrawal of IST. They recommended immediate withdrawal of IST for patients demonstrating increasing mixed chimerism (MC). They also reported the results of DLI for 21 patients with JMML who developed MC following SCT [15]. Response to DLI were observed in six patients and only one of the responders was alive and in remission for more than 1,500 days. Therefore, the role of these immunological interventions for treatment of MC in patients with JMML remains uncertain. In this retrospective study, we analyzed serial chimerism status following SCT and evaluated the efficacy of withdrawal of IST or DLI for the management of MC.

METHODS

Between February 1996 and May 2011, 16 children with JMML received SCT at the Section of Pediatrics, National Kyushu Cancer Center. The diagnosis of JMML was made according to the criteria proposed by the International JMML Working Group [16]. The patients included 12 males and four females with a median age of 14 months (range, 3–50) at the time of diagnosis. Karyotypic abnormalities were detected in three patients including a case of monosomy seven. Six patients have gene mutations including the PTPN11 mutation in three patients, RAS mutation in two and NF1 in one. No genetic abnormalities were detected in two patients and another eight patients were not examined for mutational status. All patients received various systemic chemotherapies including 6-mercaptopurine (6MP), etoposide, and cytarabine prior to SCT. Three patients received acute myelogenous leukemia-oriented induction chemotherapies. Two patients underwent splenectomy prior to SCT. Median spleen size measured in the left midclavicular line below the costal margin was 5 cm (range, 0–16) before conditioning chemotherapy prior to SCT in the remaining 14 patients. The procedures for SCT are shown in Table I. The median age was 26.5 months

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TABLE I. Procedures and Outcomes of SCT

	1st SCT (n = 16)	2nd SCT (n = 11)
Age at SCT (months)	26.5 (9–52)	41 (25–60)
Sex (M/F)	12/4	8/3
Diagnosis to SCT (months)	7.5 (2–37)	15 (10–38)
Donor type and HLA matching		
HLA-matched related	4	1
HLA-mismatched related	1	1
HLA-matched unrelated	8	4
HLA-mismatched unrelated	3	5
Stem cell source		
BM	11	4
CB	3	6
PB	2	1
Conditioning regimen		
BU/CY ± others	9	4
BU/Flu/L-PAM	4	0
TBI/CY ± others	1	2
TBI/VP16/L-PAM	0	1
Flu/L-PAM	2	4
GVHD prophylaxis		
CsA ± sMTX	6	2
FK506 ± sMTX	10	9
Acute GVHD (grade)		
0–I	3	2
II–IV	10	9
NE	3	0
Chronic GVHD		
None	5	2
Limited	2	2
Extensive	2	2
NE	7	5
Outcome		
HR	3	5
RP	6[a]	1
PD	3	3
GF	4	0
TRD	0	2

SCT, stem cell transplantation; HLA, human leukocyte antigen; BM, bone marrow; CB, cord blood; PB, peripheral blood; BU, busulfan; CY, cyclophosphamide; Flu, fludarabine; L-PAM, melphalan; VP16, etoposide; TBI, total body irradiation; GVHD, graft-versus-host disease; CsA, cyclosporine A; sMTX, short-term methotrexate; FK506, tacrolimus; NE, not evaluable; HR, hematological remission; RP, relapse; PD, persistent disease; GF, graft failure; TRD, treatment-related death. [a]Two of these six patients restored HR again after immunological interventions following RP.

(range, 9–52) at the time of SCT. Thirteen patients received a busulfan-based myeloablative conditioning regimen and one patient received a total body irradiation- (TBI) based myeloablative regimen for the first SCT. The remaining two patients received a reduced-intensity conditioning regimen consisting of fludarabine (Flu) and melphalan (L-PAM). Among these 16 patients, 11 patients received a second SCT because of disease recurrence or graft failure (GF). As a result, a total of 27 SCT were performed for 16 patients.

Engraftment was defined as the first day when the absolute neutrophil count (ANC) reached 0.5×10^9/L for three consecutive days. Both the platelets and reticulocytes were considered to be recovered when they reached counts greater than 20×10^9/L and over 1% without transfusion support. GF was defined as a lack of neutrophil recovery irrespective of donor chimerism. Patients who had MC at the time of neutrophil recovery were not considered to have achieved engraftment.

Chimerism analysis was performed when neutrophil recovery was observed or graft failure or relapse was suspected and thereafter according to the physician's discretion. Donor chimerism was evaluated using bone marrow (BM); peripheral blood (PB) samples were collected when bone marrow was not available. Chimerism analysis was examined by fluorescence *in situ* hybridization (FISH) with sex chromosomes for sex mismatched SCT and short tandem repeat polymerase chain reaction (STR-PCR) for sex matched SCT using whole BM or PB cells. The sensitivity of these methods to detect autologous cells is 5%. MC was defined as the presence of >5% autologous cells.

In this study the clinical outcomes of SCT in JMML patients were evaluated as follows. Hematological remission (HR) was defined as a persistent complete chimerism (CC) for two consecutive assessments after engraftment. Relapse (RP) was defined as an increase in the proportion of autologous cells >5% in the presence of clinical signs of JMML (including increased spleen size, thrombocytopenia or blast cells in PB or BM) with prior HR. Persistent disease (PD) was defined as MC at the time of neutrophil recovery followed by an increase in the proportion of autologous cells in the presence of clinical signs of JMML without achieving CC. Patients who had maintained HR without splenomegaly or other clinical symptoms of JMML were considered to have achieved complete remission (CR).

Withdrawal of IST was defined as follows. Discontinuation of IST was defined as stopping IST within 2 weeks after detection of MC. Tapering of IST was defined as stopping IST more than 2 weeks after detection of MC. The duration of withdrawal of IST was dependent on the clinical conditions of each case.

RESULTS

Overall Patient Outcome

The outcomes for first and second SCT are shown in Table I and in Figure 1. Following the first SCT, 12 of 16 patients (75%) had neutrophil recovery higher than 0.5×10^9/L after a median of 19.5 days (range, 13–33) and the remaining four patients had GF. Among the 12 patients who had neutrophil recovery, three patients were diagnosed as having PD soon after neutrophil recovery. Of the remaining nine patients, six relapsed between 3 and 7 months after achieving HR. Of the two patients who received reduced-intensity conditioning regimens, one had PD and the other RP. Therefore, only 3 of 16 patients had continuous HR following engraftment until the last follow-up after the first SCT. Two of six patients who relapsed after their first SCT achieved HR again after immunological intervention, which included withdrawal of IST or DLI. The remaining four patients received a second SCT. No treatment-related death was observed after the first SCT. As a result, 11 patients received second SCT because of GF in four patients, PD in three and RP in four. Among the 11 patients who received a second SCT, eight patients achieved HR and five of these eight patients are alive in remission at the last follow-up. All three patients who had PD after the first SCT developed PD again following the second SCT. Overall, 10 of 16 patients (62.5%) are alive in remission with a median follow-up of 43 months (range, 12–176) from their first SCT.

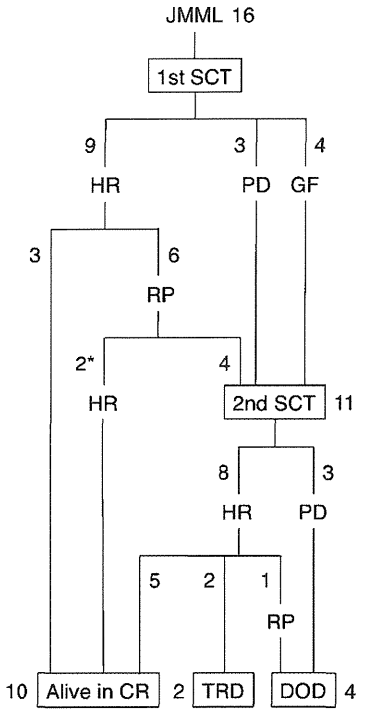

Fig. 1. Flow diagram of clinical outcome after 1st and 2nd SCT in children with JMML. SCT, stem cell transplantation; HR, hematological remission; RP, relapse; CR, complete remission; PD, persistent disease; GF, graft failure; TRD, treatment-related death; DOD, dead on disease. *Two patients restored HR after immunological interventions following RP.

Outcome of Withdrawal of IST for Mixed Chimerism

Chimerism analysis was available in 26 SCT cases following the first and second SCT. Six of 26 cases had CC in all samples and maintained remission at the time of the last follow-up. Except for four GF cases, MC was observed in the remaining 16 cases and withdrawal of IST was performed in 14 cases immediately after identification of MC. Table II shows the outcome of withdrawal of IST for MC cases according to the time at which MC developed. Eight cases had MC at the time of neutrophil recovery, with a median level of 18% autologous cells (range, 7% to ≥95%). Following withdrawal of IST, three cases (UTN 138-2, 381-1, and 382-2) achieved CC 30, 49, and 88 days after SCT and the percentages of autologous cells at the start of withdrawal of IST were 13%, 7%, and 9%, respectively. Thereafter, they maintained HR until the last follow-up. In the remaining five cases, the median proportion of autologous cells was 68% (8% to ≥95%) at the time of neutrophil recovery and the proportion of autologous cells increased rapidly although IST was immediately discontinued or tapered. All five of these cases developed PD.

Following achievement of HR, six cases developed MC and received withdrawal of IST. The median proportion of autologous cells at the detection of MC was 23% (range, 11–77%). Response to withdrawal of IST was observed in two cases. In these two cases (UTN 118-1, 402-2), MC was identified 90 and 108 days after SCT with increased autologous chimerism (25% and 50%, respectively) and CC was restored 30 and 61 days after the start of withdrawal of IST. In the remaining four cases, despite discontinuation or tapering of IST, the proportion of autologous cells increased. All four of these cases developed RP. Overall, withdrawal of IST was efficacious in 5 of 14 cases. One of these five cases relapsed 44 days after restoration of CC and the remaining four cases were in CR at the time of the last follow-up.

GVHD and Donor Chimerism After Withdrawal of IST

Following withdrawal of IST, six of eight MC cases at the time of neutrophil recovery developed grade II–IV acute GVHD (Table II). Three cases achieved CC after developing grade III–IV acute GVHD and one case (UTN 138-2) died of grade IV acute GVHD. In the remaining three cases, the proportion of autologous cells increased rapidly despite the occurrence of acute GVHD, grades II–IV. They were all diagnosed as PD. UTN 173-2 had PD

TABLE II. Outcome of Withdrawal of IST for Mixed Chimerism Following SCT

UTN	Detection of MC	% autologous cells (days)	Withdrawal of IST[a]	After withdrawal of IST		Outcome (days)
				GVHD	% autologous cells (days)	
138-2	At neut. recovery	13 (14)	d/c	IV	<5 (30)	HR
381-1	At neut. recovery	7 (21)	Tapering	III	<5 (49)	HR
382-2	At neut. recovery	9 (21)	Tapering	III	<5 (88)	HR
173-1	At neut. recovery	≥95 (37)	d/c	II	≥95 (50)	PD
173-2	At neut. recovery	68 (26)	Tapering	IV	≥95 (58)	PD
305-1	At neut. recovery	≥95 (18)	d/c	—	NE	PD
310-1	At neut. recovery	23 (26)	d/c	III	≥95 (60)	PD
310-2	At neut. recovery	8 (19)	d/c	I	52 (60)	PD
118-1	Following HR	25 (90)	Tapering	—	<5 (120)	HR
402-2	Following HR	50 (108)	Tapering	III	<5 (169)	HR
239-1	Following HR	14 (172)	d/c	—	22 (366)	RP
256-1	Following HR	11 (124)	d/c	I	≥95 (205)	RP
309-1	Following HR	77 (162)	d/c	—	81 (188)	RP
382-1	Following HR	21 (87)	d/c	III	85 (119)	RP

IST, immunosuppressive therapy; SCT, stem cell transplantation; UTN, unique transplantation number; MC, mixed chimerism; GVHD, graft-versus-host disease; neut., neutrophil; d/c, discontinuation; NE, not examined; HR, hematological remission; PD, persistent disease; RP, relapse.
[a]Tapering means stopping IST within 2 weeks after detection of MC and d/c means stopping IST more than 2 weeks after detection of MC.

and died of progressive JMML, although he developed grade IV acute GVHD.

In six MC cases following achievement of HR, two cases developed grade III acute GVHD following withdrawal of IST. One case (UTN 402-2) achieved CC but the other case (UTN 382-1) did not respond despite the occurrence of acute GVHD. UTN 118-1 was restored to CC following interventions without acute GVHD.

Among five cases who responded to withdrawal of IST, four cases developed extensive chronic GVHD after achievement of HR and three cases are alive in remission at the last follow-up. Overall, 8 of 10 survivors had chronic GVHD (limited type in four patients and extensive in four).

Outcome of DLI

Five cases received DLI without receiving chemotherapy prior to DLI (Table III). A response was observed in only one patient (UTN 239-1) who received DLI of 2.1×10^7/kg T cells from a matched unrelated donor when the proportion of autologous cells was 22%. CC was achieved again 3 months after DLI without occurrence of acute or chronic GVHD and he remains in remission with a follow-up of 96 months after SCT. Although UTN 382-1 developed grade II acute GVHD following DLI, the proportion of autologous cells increased.

DISCUSSION

In this study, MC was observed in about two-thirds of SCT cases in children with JMML. This observation supports the results of a previous report in which MC was noted in 16 out of 30 patients, indicating that MC is not an uncommon phenomenon after SCT for JMML [14]. It is noteworthy that almost half of the episodes of MC were detected at the time of neutrophil recovery in our study. Three of eight MC cases at the time of neutrophil recovery achieved CC following withdrawal of IST. The proportion of autologous cells at the time of neutrophil recovery was around 10% in the three successful cases, which suggests that early detection of MC is critical and examination of chimerism immediately after neutrophil recovery is recommended.

In our study, a high incidence of PD (23%) was observed after SCT. To our knowledge, however, the conditions that we defined as PD have not been emphasized in recent studies of SCT in JMML [4–9]. We consider that there are two possible explanations. First, in previous studies, similar patients might have been diagnosed as early relapse when analysis of donor chimerism was not available. Second, the condition might have been referred to as graft rejection. In our study, all three patients who had PD after their first SCT repeated the same episode after a second SCT. Although the reason is unclear, we postulate that our patients who experienced PD might have been refractory to the conditioning chemotherapy and the original disease might have recurred rapidly after SCT.

Among 14 MC cases reported here, five cases achieved CC following withdrawal of IST. In the five cases who received DLI, CC was restored in one case. Although these observations suggest the presence of a GVL effect in SCT with JMML, only a limited number of patients have the benefit of these treatment modalities as previously described [14,15]. In addition, our study demonstrated that the proportion of autologous cells at the time of interventions is not necessarily associated with treatment response. The limitation of this study is that it was a retrospective analysis and the number of patients is too small thus far to draw definitive conclusions. However, because early detection of MC could make possible early interventions, which may be beneficial for some patients, serial and periodic analyses of chimerism should be further studied after SCT in children with JMML. The other limitation of this study is that the sensitivity of chimerism analysis is 5%, which is relatively low compared to recently reported methods [17]. Further progress and standardization of techniques to detect autologous cells in chimerism analysis should be made.

Whether there is a role for acute GVHD in the induction of the GVL effect after intervention is uncertain. Here, six of eight cases who had MC at the time of neutrophil recovery developed grade II–IV acute GVHD following withdrawal of IST. Of these, three cases achieved CC after development of acute GVHD, one died from grade IV acute GVHD, while the remaining three cases showed PD despite the occurrence of severe acute GVHD. In six cases who had MC following HR, two cases developed grade III acute GVHD and one of them achieved CC again. Other successful cases following interventions did not develop acute GVHD. Therefore, acute GVHD may not always be associated with a GVL effect associated with achievement of CC. We have to consider the risk of development of life-threatening acute GVHD by withdrawal of IST and the possibility that CC could be achieved without intervention. Further large scale studies with close monitoring of chimerism and GVHD are required to estimate the efficacy and safety of early intervention.

In our study, most of the successfully sustained remission cases developed chronic GVHD. On the other hand, a total of

TABLE III. Outcome of DLI

UTN	Donor (HLA disparity)	Withdrawal of IST	SCT-DLI (months)	Autologous cells at DLI (%)	No. of DLIs	Total no. of infused $CD3^+$ cell (10^7/kg)	GVHD after DLI	Outcome
239-1	UBM (6/6)	Ineffective	11	22	1	2.1	—	HR
138-1	RBM (6/6)	Not given	7	59	1	26	—	RP
309-1	RBM (6/6)	Ineffective	6	81	1	1.7	—	RP
310-2	UBM (6/6)	Ineffective	2	52	1	2.5	—	PD
382-1	RBM (6/6)	Ineffective	3.5	21	2	7.2	II	RP

DLI, donor lymphocyte infusion; UTN, unique transplantation number; IST, immunosuppressive therapy; UBM, unrelated bone marrow; RBM, related bone marrow; SCT, stem cell transplantation; GVHD, graft-versus-host disease; HR, hematological remission; RP, relapse; PD, persistent disease.

seven RP occurred after the first and second SCT, and five cases had no chronic GVHD. In addition, spleen size decreased gradually and normalized several months after engraftment without additional cytotoxic treatment in those sustained remission cases that had splenomegaly before SCT. These observations strongly suggest continuous tumor eradication by donor cells, which caused a lasting GVL effect. Overall, GVL seems to play an essential role in preventing the recurrence of JMML. In our study, the incidence of acute and chronic GVHD was high probably because cyclosporine A or tacrolimus were tapered or discontinued soon after SCT for the purpose of deriving a GVL effect reflecting the high incidence of MC. However, given the morbidity and mortality of severe acute and chronic GVHD, further investigation will be required to establish the optimal strategy to induce a GVL effect without severe GVHD. Long-term follow-up is also needed to estimate the effect of chronic GVHD for the quality of life.

Generally, the outcomes of second SCT in patients with hematological malignancies who relapsed after the first SCT are dismal [19,20]. However, for JMML in children, the results of a second SCT are reportedly similar to those obtained with the first SCT [18,21]. In our study, 11 patients with JMML received a second SCT and five of them are alive in remission with a median follow-up of 48 months. This result would support the feasibility of a second SCT for JMML. However some patients could achieve remission by immunological interventions without a second SCT. Close monitoring of chimerism and early detection of MC is feasible to assist treatment decisions after SCT in children with JMML. If immunological interventions are ineffective, a second SCT should be considered.

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The second therapeutic trial for children with hematological malignancies who relapsed after their first allogeneic SCT: Long-term outcomes

Nishikawa T, Inagaki J, Nagatoshi Y, Fukano R, Nakashima K, Ito N, Sawa D, Kawano Y, Okamura J. The second therapeutic trial for children with hematological malignancies who relapsed after their first allogeneic SCT: Long-term outcomes.

Abstract: The impact of a second all-SCT on the long-term outcomes of children who relapse after allo-SCT has been unclear. We retrospectively analyzed the long-term outcomes of different salvage treatments for such children. Sixty-six children with hematological malignancies (40 ALL, 22 AML, three MDS, and one CML) who relapsed after a first allo-SCT received either a second allo-SCT (n = 16) or CTx and/or DLI (n = 50). The median follow-up for all children was 9.1 yr. The five-yr OS after relapse was significantly better in patients who underwent a second allo-SCT (42.9%) than in patients treated with CTx and/or DLI (11.8%) ($p < 0.05$). However, this advantage diminished with increasing time. The eight-yr OS for these groups of patients were 21.4% and 11.8%, respectively (p = n.s.). Among the 16 patients who received a second allo-SCT, two died more than five yr after the second allo-SCT. A second allo-SCT can therefore lead to a prolonged OS in patients who relapse after allo-SCT. However, a second allo-SCT should be selected carefully. This is because the mortality rate is still high, even when there is an extensive duration of time following the second allo-SCT.

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A relapse of disease is the most common cause of treatment failure in children with hematological malignancies undergoing allo-SCT (1). The prognosis for children with relapse after allo-SCT is still extremely poor (2–5), and the optimal treatment for children who relapse after allo-SCT is not yet established. The treatment options range from intensive treatments, including re-induction CTx, DLI, and a second allo-SCT, to simple palliative care in some cases (6). DLI can induce remission in patients with CML (7), but the role of DLI in the management of other

Abbreviations: 6-MP, 6-mercaptopurine; ALL, acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; AML, acute myeloid leukemia; AVN, avascular necrosis; BCP-ALL, B-cell precursor ALL; BM, bone marrow; BO, bronchiolitis obliterans; BU, busulfan; Bu, busulfan; CA, cytarabine; CB, cord blood; CI, confidence intervals; CML, chronic myeloid leukemia; CP, chronic phase; CR, complete remission; CsA, cyclosporine; CTx, chemotherapy; DLI, donor lymphocyte infusion; EBMT, European Group for Blood and Marrow Transplantation; GETMON, Spanish Working Party for Blood and Marrow Transplantation in Children; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; L-PAM, melphalan; MDS, myelodysplastic syndrome; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; OS, overall survival; PBSC, peripheral blood stem cell; PD, progressive disease; Ph-ALL, Philadelphia chromosome–positive ALL; PS, performance status; RIC, reduced-intensity conditioning; RP, relapse; sMTX, short-term methotrexate; Tac, tacrolimus; T-ALL, T-cell ALL; TBI, total body irradiation; TRM, treatment-related mortality; UPN, unique patient numberUPN, unique patient number.

pediatric hematological malignancies that relapse after allo-SCT is poorly understood, and studies have been limited to case reports and small case series (8).

Several studies have shown that a second allo-SCT improved the survival after relapse and that it represents a potential therapeutic option, which may increase the duration of disease-free survival (9–13). However, the long-term outcome of the entire population of children who experienced a relapse after allo-SCT, including those who received a second allo-SCT, has not yet been determined (14, 15).

We performed a retrospective single-institute analysis of children with ALL, AML, MDS, or CML who relapsed after a first allo-SCT to compare the long-term outcomes for relapsed disease treated with second allo-SCT vs. CTx and/or DLI. We herein present the long-term follow-up data on this consecutive series of children and discuss the effects and limitations of a second allo-SCT.

Methods

Patients

From August 1983 to December 2010, a total of 250 patients with leukemia or MDS underwent allo-SCT at the National Kyushu Cancer Center. The characteristics of the patients and the transplantations are summarized in Table 1. The underlying diseases were ALL (n = 132), AML (n = 68), MDS (n = 33), and CML (n = 17). The median age at the first allo-SCT was eight yr (range, 0–24). All patients received a first allo-SCT with myeloablative conditioning. All grafts were 5/6 or 6/6 HLA-antigen matched from related or unrelated donors.

Table 1 Patient and transplantation characteristics

Characteristic	All patients	Relapsed patients %
No. of patients	250	71 (28)
Age, years, median (range)	8 (0–24)	10 (0–18)
Diagnosis		
ALL	132	43 (33)
AML	68	22 (32)
MDS	33	4 (12)
CML	17	2 (12)
Gender		
Male	143	45 (31)
Female	107	26 (24)
MRD		
Yes	97	32 (33)
No	153	39 (25)
Conditioning regimen		
TBI-based	179	54 (30)
Bu-based	63	15 (24)
Other	8	2 (25)
Stem cell source		
BM	213	60 (28)
PBSC	4	1 (25)
CB	33	10 (30)
Disease status at first SCT		
CR	187	45 (24)
Non-CR	63	26 (41)
GVHD prophylaxis		
CsA-based	122	41 (34)
Tac-based	95	22 (23)
MTX	30	6 (20)
Others	3	2 (66)

Definitions

Relapse after allo-SCT was defined as the presence of or an increase in leukemic blasts as detected by morphology in either the BM or peripheral blood. For Ph-ALL, CML, and MDS, the presence of the clonotypic molecular or cytogenetic abnormality was sufficient to diagnose relapsed or refractory disease.

Post-relapse treatments were categorized into two cohorts: cohort 1 received re-induction CTx and/or DLI, and cohort 2 received a second allo-HCT. The treatments were decided at a multiprofessional conference, at which the clinical circumstances and the opinions of physicians and the patient or their parents were weighed. CTx included re-induction with regimens containing CA, anthracycline, etoposide, steroid, methotrexate, 6-MP, L-asparaginase, nelarabine, vincristine, or any combinations thereof. Imatinib mesylate was administered for Ph-ALL and CML.

The response to post-relapse salvage therapy and post-relapse survival was evaluated for each salvage modality. CR was defined as normocellular BM with less than 5% blasts, along with the absence of blasts in the peripheral blood (16). Post-relapse survival was either measured from the time of relapse to the time of death or censored at the last contact date if the survival status was unknown. TRM was defined as death from toxicities related to therapy without disease recurrence.

Statistical analysis

The statistical analyses were performed using the SPSS software program version 19.0 (SPSS Co., Tokyo, Japan). Surviving patients were followed until December 31, 2010. The OS was calculated by the Kaplan–Meier method, with 95% CI. The outcomes of patients who did or did not receive a second allo-SCT were compared after adjusting for the waiting time until the second allo-SCT. The patients who died before the median time to

the second allo-SCT (six months) were excluded from this comparison. Differences between groups were evaluated using a log-rank test. The multivariate Cox proportional hazard model was applied to analyze the risk factors regarding death after post-allo-SCT relapse. A probability value of <0.05 was considered to be statistically significant.

Results

Relapse

The characteristics of all patients and relapsed patients are shown in Table 1. Overall, 71 of the 250 patients (28%) relapsed at a median of 241 days (range, 20–7455) after the first allo-SCT (43 ALL, 22 AML, four MDS, and two CML). The interval from the first allo-SCT to relapse was less than 100 days in 20 patients.

Post-relapse treatments

The various therapeutic options used after the diagnosis of relapse are summarized in Fig. 1. When a relapse of primary disease was diagnosed, immunosuppressive treatment was discontinued in all patients in the absence of significant GVHD. Discontinuation of immunosuppressive drugs induced remission in two children (one MDS patient with a relapse of a chromosomal abnormality and one CML patient with a molecular relapse), both of whom are still alive and have been in continuous remission. Three patients with ALL received supportive care only, with no cytotoxic agents, because of either their poor clinical condition or a refusal to undergo any further treatments, and they all died of leukemia. Excluding these five patients, a total of 66 cases were analyzed.

These 66 patients received CTx only (n = 45), DLI only (n = 2), DLI and CTx (n = 3), or a second allo-SCT (n = 16) as treatment for their relapse. The characteristics of children who received a second allo-SCT or other treatments without a second allo-SCT are shown in Table 2. In the group that received CTx and/or DLI (cohort 1), 50 of the 66 patients relapsed at a median of 227 (range, 20–1630) days after the first allo-SCT; these patients received CTx and/or DLI. Half of these patients received their first allo-SCT in non-CR. In the group that received a second allo-SCT (cohort 2), 16 of the 66 (24.2%) patients had a median relapse time of 490 (range, 75–1833) days after the first SCT; these patients were given a second allo-SCT. These patients were in good clinical condition and had an appropriate donor for allo-SCT, but no specific eligibility criteria existed. The disease status at the time of the second allo-SCT was as follows: 11/16 patients were in CR or CP, and 5/16 had either a RP or PD. All patients received a conventional myeloablative conditioning regimen (Table 3). Four patients received allo-SCT from the same donor as in the first allo-SCT (related BM donor, n = 3; related PBSC, n = 1), and the remaining 12 received the second allo-SCT from a different donor (related BM

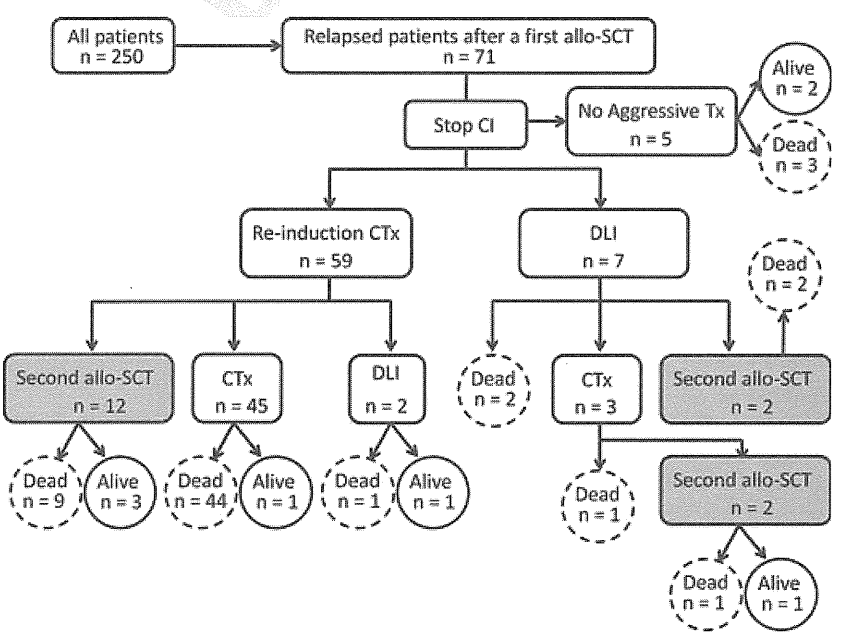

Fig. 1. Summary of the therapies administered after relapse.

Table 2 Characteristics of patients in the treatment group

	CTx and/or DLI	Second allo-SCT	p
No. of patients	50	16	
Median age at first allo-SCT (yr)	9 (0–18)	11 (3–16)	
Diagnosis			
ALL	33	7	007
AML	16	6	
MDS	1	2	
CML	0	1	
Disease status at first allo-SCT			
CR	25	14	<001
non-CR	25	2	
Time from first allo-SCT to relapse			
>100 days	36	13	047
<100 days	14	3	
Years of RP after first allo-SCT			
1983–2001	26	8	077
2001–2010	24	8	

Table 3 Transplant-related data of patients who received a second allo-SCT

	At first SCT	At second SCT
Conditioning regimen		
TBI-based	12	4
Bu-based	4	12
Donor and stem cell source		
Related donor		
BM	11	4
PBSC	0	1
Same donor as first SCT		4
Unrelated donor		
BM	3	10
PBSC	1	0
CB	1	1
HLA		
Matched	12	11
Mismatched	4	5
GVHD prophylaxis		
CsA/sMTX or CsA	11	6
Tac/sMTX or Tac	3	9
MTX	2	1

donor, n = 1; unrelated BM donor, n = 10; CB, n = 1).

Nine patients (three ALL, five AML, and one MDS), who relapsed at a median of 236 (range, 31–1167) days after allo-SCT, received DLI from their first allo-SCT donor containing a median of 5×10^7 donor T cells/kg (administered a median number of two times); two of these nine patients received DLI after induction CTx, according to the schema developed by Levine et al. (17), and four of these nine patients received a second allo-SCT as an additional salvage treatment.

Survival

The CR rate, five-yr OS rate, and median survival duration for all 66 children were 37.9%, 12.1% (4.3–19.9), and 164 days (range, 11–5040). While the probabilities of achieving CR and the median post-relapse survival duration were 75% and 540 days (range, 100–3431), respectively, for the 16 children who received a second allo-SCT, they were 26% and 130 days (range, 11–5040) for the 50 children who received CTx and/or DLI. There was a significant difference in the five-yr OS even after adjusted for the waiting time until the second allo-SCT, between the 14 patients (42.9%) who received a second allo-SCT and the 17 patients (11.8%) who received CTx and/or DLI ($p < 0.05$) (Fig. 2). Of these 17 patients in cohort 1, 2 received both DLI and CTx.

After a median follow-up of 9.1 yr in all children, the eight-yr OS was 21.4% for patients who received a second allo-SCT. The difference between the second allo-SCT and CTx and/or DLI cohorts became nonsignificant at this time. Even after five yr, the Kaplan–Meier curve of the second allo-SCT cohort did not reach a plateau (Fig. 2). While two of the 50 patients who received CTx and/or DLI without a second allo-SCT have survived long-term, two of the 16 patients in cohort two died more than 5 yr after the second allo-SCT. Patient 217 died because of BO, and another patient (125) died because of a recurrence of leukemia. The detailed clinical courses of the long-term survivors who relapsed after SCT are shown in Table 4. Both patients 101 and 186 were scheduled to undergo a second allo-SCT, but this plan was abandoned because of organ dysfunction. Patient 101 received a combination of mild CTx, 6-MP, and methotrexate and thereafter has been in a state of remission. Patient 186 developed osteosarcoma in the pelvis 10 yr after SCT and is currently receiving CTx.

In the second allo-SCT and CTx and/or DLI cohorts, the TRM were 50% (25.5–74.5) and 8% (0.6–15.4), respectively (8/16, 4/50). The main cause of death in these cohorts was primary disease in 48 (4/16, 44/50), followed by infection in six patients (2/16, 4/50). GVHD was the main cause of death in four patients (4/16, 0/50), and veno-occlusive disease was the cause of death in two patients (2/16, 0/50), all of whom had received a second allo-SCT.

When we focus on the analysis of patients with ALL who received their first allo-SCT in CR1–3 (n = 24), there was a still significant difference in the eight-yr OS between the seven patients (28.6%, 0–62.1) who received a second allo-SCT and the 17 patients (5.9%, 0–17.1) who received CTx and/or DLI ($p < 0.05$). A multivariable analysis showed that a longer time to

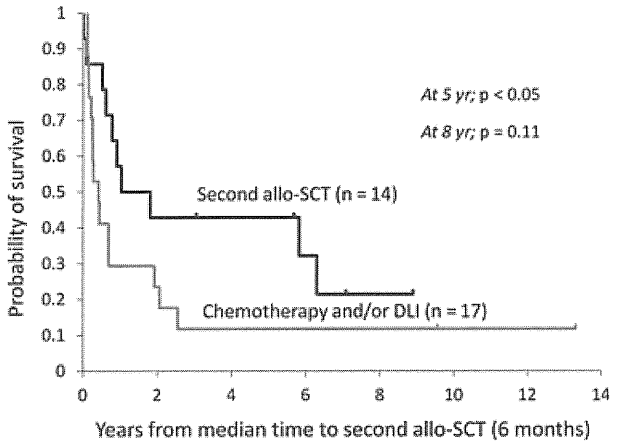

Fig. 2. The OS of patients who relapsed after the first allo-SCT stratified by the treatment performed and adjusted for the waiting time prior to the second allo-SCT.

post-first allo-SCT relapse and second allo-SCT appeared to be associated with an improved post-relapse survival (Table 5).

In the children who received DLI treatment, two children with hematological relapse who received CTx followed by DLI achieved remission. One (ALL) survived in remission for 10 yr, while the other (AML) relapsed and died within six months. Six patients with hematological relapse and one with cytogenetic relapse did not respond to DLI. Salvage therapy with a second transplant was successful in one of four patients.

Discussion

The relapse of hematological malignancy after allo-SCT remains a significant therapeutic challenge and is the main cause of treatment failure after allo-SCT. Because of the increase in the number of children who receive allo-SCT, studies on the long-term outcome of relapse after allo-SCT are needed. For this purpose, we retrospectively analyzed the treatments for such children and their long-term outcomes.

Our study emphasizes the grim prognosis for a relapse of hematological malignancy after allo-SCT and highlights the need for more effective therapies. While the treatment groups in our study were biased by the different therapeutic decisions at the time of relapse, our observations suggest that a second allo-SCT may be beneficial for obtaining an improvement in post-relapse survival. The median duration of the post-relapse survival was longer for patients receiving a second allo-SCT than for those not receiving a second allo-SCT in our study. Arellano et al. (14) also reported the outcomes of 100 adult patients with acute leukemia who relapsed after allo-SCT.

There have so far been few reports about the outcomes of a second allo-SCT in pediatric patients. A study by the EBMT reported a three-yr OS of 33% for childhood patients with ALL who received a second allo-SCT (10), while the GETMON reported an event-free survival of 32% at five yr for childhood with hematological malignancies (5). However, they did not report on any patients with a longer-term outcome than five yr. Furthermore, their studies have not compared the outcome of a second allo-SCT with that of other interventions.

Although a second allo-SCT may be beneficial for children, it could be applied for only a minority of patients in our cohort (16 of 66 children) because of disease progression, severe complications, and graft availability. Recently, several studies have reported favorable outcomes in patients who received a second allo-SCT using a RIC regimen (18, 19). Although RIC regimens administered prior to a second allo-SCT may expand the applicability of this modality in the relapse setting, information regarding the long-term outcome of such a treatment is limited (18, 19). All of the second allo-SCTs in our study were performed using a myeloablative conditioning regimen; however, the use of an RIC regimen might be an alternative for patients with a poor PS (measured by the Eastern Cooperative Oncology Group criteria).

It is worth mentioning that two patients in our study died more than five yr after the second allo-SCT. One patient died because of TRM, and another died because of a recurrence of the primary disease. Attention should therefore be paid to TRM and the recurrence of the primary disease even after a long period of time. Kurosawa et al. also showed improvement in the one-yr OS for adult patients with leukemia who received a second allo-SCT. However, at two yr, the difference between the second allo-SCT and CTx and/or DLI cohorts became smaller and nonsignificant (15). They suggested that the second allo-SCT may produce improved survival, but the substantial incidence of a later relapse after the second allo-SCT was revealed to be a major concern. When analyzing only patients with ALL who received their first allo-SCT in CR1–3 in our study, a second allo-SCT is more beneficial for a positive long-term outcome than in patients with a poorer status.

Of the four patients who received a second allo-SCT and survived for a long period of time in the present study, three were in CR2 at the second allo-SCT. The disease state at the second allo-SCT seemed to be a determining factor in

Table 4 Characteristics of long-term survivors who relapsed after first allo-SCT

UPN	Age at first SCT	Sex	Diagnosis	Disease state at first SCT	Conditioning regimen (first SCT)	Donor/stem cell source (first SCT)	Days from first SCT to relapse	Disease status at second SCT	Conditioning regimen (second SCT)	Donor/stem cell source (second SCT)	Late complication	Current PS	Interval from relapse to last follow-up (yr)
101	12	F	BCP-ALL	CR3	TBI/L-PAM	MUD/BM	605	–	–	–	–	1	138
186	13	F	BCP-ALL	IF	TBI/CA/CY	MRD/BM	236	–	–	–	Osteosarcoma	4	101
226	15	M	T-ALL	CR1	TBI/CA/CY	MRD/BM	132	CR2	BU/L-PAM	MRD/PBSC	AVN	1	94
247	7	F	AML	CR1	BU/CY	MRD/BM	685	CR2	TBI/CY	MUD/BM	–	1	62
276	14	M	Ph-ALL	CR1	TBI/CA/CY	MRD/BM	473	CR2	BU/CY	MMUD/BM	AVN	0	76
354	12	M	AML	CR2	TBI/CY	MRD/BM	313	CR3	BU/L-PAM	MUD/BM	–	1	36
125	12	M	BCP-ALL	CR2	TBI/L-PAM	MUD/BM	1833	CR3	BU/CY	MUD/BM	Relapsed disease (Dead)	–	63
217	15	M	AML	RP	TBI/CY	MUD/BM	94	RP	BU/CY	MUD/BM	BO (Dead)	–	68

BU (16 mg/kg), CA (3 g/m^2 × 4), CY (60 mg/kg × 2), L-PAM (90 mg/m^2 × 2), TBI (12 Gy).

Table 5 Factors associated with longer post-relapse survival

Factor	p Value	HR (95% CI)
Disease status at first SCT (CR)	0.54	–
Duration from first SCT to relapse ≥6 months	0.04	0.58 (0.34–0.98)
Second SCT	0.01	0.32 (0.17–0.62)

*HR <1 was associated with longer post-relapse survival.

the outcome of the second allo-SCT, similar to that proposed in other reports (9–13).

With regard to the donor of the second allo-SCT, if there is a different HLA-matched sibling or unrelated donor and no antecedent GVHD, this donor could be considered to enhance graft-versus-leukemia reactivity. However, this effect has not yet been conclusively proven and will therefore need to be examined in further studies (4, 6).

The incidence of TRM caused by a second allo-SCT was extremely high. In our study, the patients who received a second allo-SCT did not have a poorer PS than those who received a single allo-SCT (Table 4). This may have been due to the individual patient conditions, because single allo-SCT cases may have had a poorer PS that prevented them from receiving the second allo-SCT.

The ability of DLI to produce durable remission for relapsed hematological malignancies was not demonstrated in our cohort. Among the nine children who received DLI, only 2 (22%) achieved CR. These remissions were short-lived, and there was only one long-term survivor. In addition, these two patients received DLI with preceding CTx; the other seven patients received DLI alone and did not respond to the treatment. Other investigators have reported similar results (7, 20, 21).

Our study is limited by several inherent selection biases. Most importantly, this was a retrospective study that compared the outcomes of interventions that were chosen at the discretion of physicians. Larger prospective multicenter studies are therefore necessary to determine whether a second allo-SCT to treat relapse should be performed in theory. However, it is very difficult to plan for this due to the fact that most physicians believe relapsed patients could not be cured by CTx alone. In addition, changes in the care of children with hematological malignancies (e.g., treatment for disease, supportive care, examination of HLA typing, and prophylaxis for acute GVHD) might have also had an impact on these results. Nevertheless, the present data were a consecutive case series from a single center that reviewed various interventions

after relapse, which allowed us to identify the factors that influenced the prognosis of patients with relapse after allo-SCT.

To the best of our knowledge, this is the first report to describe the long-term outcomes in a pediatric population who experienced a relapse after allo-SCT. In conclusion, the long-term outcomes of children who relapsed after allo-SCT were very poor, and most patients ultimately died of their disease. A second allo-SCT can lead to prolonged OS in patients who experienced a relapse after allo-SCT. However, the mortality rate is still high, even after an extensive duration of time has passed following the second allo-SCT. Furthermore, a few patients who relapse after allo-SCT survive long term without a second allo-SCT. This is why a second allo-SCT should be carefully considered while taking into account such factors as the patient's disease status, PS, and the time to relapse.

Conflict of interest

The authors have no conflicts of interest to declare.

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LETTER TO THE EDITOR

Successful treatment with plasma exchange for disseminated cidofovir-resistant adenovirus disease in a pediatric SCT recipient

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Adenovirus (AdV) infections are a frequent cause of morbidity and mortality following allo-SCT, and disseminated infection is associated with a high rate of mortality (20–60%),[1–3] particularly in pediatric SCT patients.[1,3] Although antiviral agents are commercially available, of which cidofovir is the most effective, treatment with cidofovir is not always effective against disseminated-AdV infections.[4–6] In some reports, clearance of the virus occurred only when the lymphocytes recovered after SCT.[7] To help accelerate the lymphocyte recovery, tapering the immunosuppressant therapy should be attempted.[8] However, this is not possible in most cases because of the development of acute GVHD, or even when it is possible, lymphocyte recovery does not always occur. We herein reported a case that developed disseminated-AdV disease after allo-SCT and was successfully treated by plasma exchange (PE) even under lymphopenic conditions.

A 2-year-old boy with ALL in relapse underwent allogeneic BMT from an human leukocyte Ag-matched unrelated donor. The conditioning regimen consisted of fractionated TBI (2 Gy × 6), etoposide (60 mg/kg) × 1 day and CY (60 mg/kg) × 2 days. Tacrolimus and short-term MTX were administered for the prophylaxis of acute GVHD. On day 19 after SCT, myeloid engraftment (ANC >500 cells/μL) was achieved. However, the patient developed grade-III (skin 3, gut 2, liver 0) acute GVHD on day 9, which was barely resolved with 2 mg/kg/day prednisolone and additional administration of MTX (10 mg/m^2, on day 28, 31, 35, 42, 51).

On day 19, the patient suddenly developed painful, gross hematuria with persistent fever despite broad-spectrum antibiotic therapy. Abdominal ultrasonography and computed tomographic scans showed the development of bilateral hydronephrosis. As adenovirus 11 was isolated from his urine, which was confirmed by PCR, treatment with cidofovir (i.v. at 1 mg/kg 3 times a week) in combination with probenecid was initiated on day 29. From day 31, the patient's total lymphocyte count was decreased to less than 3×10^2 cells/μL. Although the administration of cidofovir was continued, his hematuria persisted, and his renal function deteriorated. Detection of AdV by RQ-PCR was positive in the plasma (1.5×10^4 copies/mL) on day 35. No other viruses (CMV, EBV and HSV) were detected. Prednisolone was tapered to a final dose of 0.8 mg/kg/day in an attempt to alleviate the immunosuppressive state, but the lymphocyte count did not increase.

On day 63, he developed impairment of consciousness, incoherent speech and motor weakness. A magnetic resonance imaging of the brain showed multiple micro-hemorrhages and cytotoxic edema detected in the brain stem, thalamus and posterior limb of the internal capsule. A cerebrospinal fluid analysis revealed elevated levels of protein (215 mg/dL) and myelin basic protein (305 pg/mL), but the cell count (2 cells/μL) was not elevated. The cerebrospinal fluid was positive for AdV as determined by RQ-PCR (1.3×10^3 copies/mL). The patient's plasma AdV load was increased further (5.9×10^5 copies/mL) despite continuation of cidofovir. Accordingly, we diagnosed him to have acute encephalitis associated with AdV infection.

The laboratory data showed that his blood urea nitrogen level was 126.5 mg/dL and his serum creatinine level was 2.6 mg/dL and, as a result, continuous hemodiafiltration was started on day 63. PE was started to remove the overproduced inflammatory cytokines and AdV on day 65. Prompt clinical improvement was observed following the first session of PE, with resolution of the fever, recovery of lymphocytopenia and a temporal improvement in renal failure. The total lymphocyte count increased rapidly up to 1061 cells/μL on day 68. The plasma AdV load, as assessed by RQ-PCR, decreased to 3.1×10^3 copies/mL 1 week after a single session of PE, and was undetectable (<100 copies/mL) 1 month later. The liquor AdV load was also undetectable (<100 copies/mL) 1 month later (Figure 1). Although the patient suffered severe neurological damage and had to be maintained on hemodialysis due to persistent renal failure, he is still alive and in continuous hematological remission.

The reason that the very high plasma viral load persisted despite a continuation of cidofovir might be that the patient had become profoundly lymphopenic (Figure 1). One possible explanation for his severe lymphocytopenia could be due to the fact that prednisolone and MTX successfully suppressed the acute GVHD, but these treatments also resulted in profound immunosuppression. Another reason was considered to be that AdV itself could induce lymphocytopenia. A delayed lymphocyte recovery, especially, a lymphocyte count less than 300 cells/μL, has been identified as a risk factor for disseminated AdV disease.[6,7]

We speculate that PE therapy might remove the AdV directly, and significantly reduce the humoral mediators which suppressed the lymphocytes. Although the effect is temporary, PE therapy dramatically decreases the virus load in a very short time.[9,10] In our case, as the lymphocyte count recovered

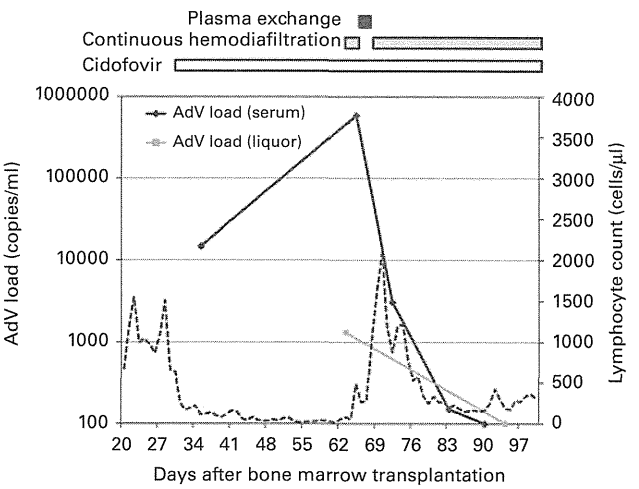

Figure 1. The clinical course of the case. Resolution of the disseminated-AdV disease was eventually achieved, with recovery of the lymphocyte count, prompted by PE. Dotted line represents lymphocyte count (cells/μL).

immediately after PE therapy, the AdV was therefore considered to have been eradicated. The role of cidofovir therapy in this patient was difficult to elucidate as the patient's condition continued to deteriorate despite receiving treatment until PE therapy was performed.

To the best of our knowledge, this is the first report to describe the effect of PE therapy on cases presenting with disseminated-AdV disease. Our experience suggests that PE might be a therapeutic tool for treating disseminated AdV refractory to cidofovir therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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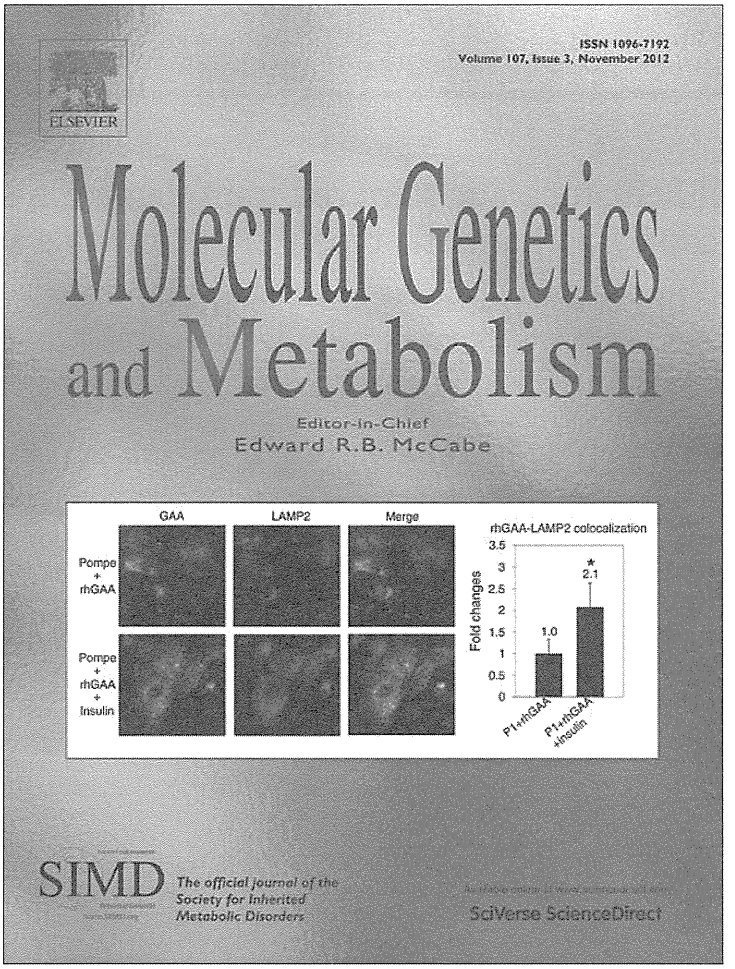

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Long-term efficacy of hematopoietic stem cell transplantation on brain involvement in patients with mucopolysaccharidosis type II: A nationwide survey in Japan

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) has not been indicated for patients with mucopolysaccharidosis II (MPS II, Hunter syndrome), while it is indicated for mucopolysaccharidosis I (MPS I) patients <2 years of age and an intelligence quotient (IQ) of ≥70. Even after the approval of enzyme replacement therapy for both of MPS I and II, HSCT is still indicated for patients with MPS I severe form (Hurler syndrome). To evaluate the efficacy and benefit of HSCT in MPS II patients, we carried out a nationwide retrospective study in Japan. Activities of daily living (ADL), IQ, brain magnetic resonance image (MRI) lesions, cardiac valvular regurgitation, and urinary glycosaminoglycan (GAG) were analyzed at baseline and at the most recent visit. We also performed a questionnaire analysis about ADL for an HSCT-treated cohort and an untreated cohort (natural history). Records of 21 patients were collected from eight hospitals. The follow-up period in the retrospective study was 9.6 ± 3.5 years. ADL was maintained around baseline levels. Cribriform changes and ventricular dilatation on brain MRI were improved in 9/17 and 4/17 patients, respectively. Stabilization of brain atrophy was shown in 11/17 patients. Cardiac valvular regurgitation was diminished in 20/63 valves. Urinary GAG concentration was remarkably lower in HSCT-treated patients than age-matched untreated patients. In the questionnaire analysis, speech deterioration was observed in 12/19 patients in the untreated cohort and 1/7 patient in HSCT-treated cohort. HSCT showed effectiveness towards brain or heart involvement, when performed before signs of brain atrophy or valvular regurgitation appear. We consider HSCT is worthwhile in early stages of the disease for patients with MPS II.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a standard therapy for young patients with mucopolysaccharidosis I (MPS I, Hurler syndrome, OMIM 607014) [1–4]. HSCT is indicated when MPS I patients are <2 years of age and show an intelligence quotient (IQ) of ≥70. However, HSCT has not been indicated for patients with mucopolysaccharidosis II (MPS II, Hunter syndrome, OMIM 309900) as no obvious efficacy has been shown on the brain involvement of MPS II patients [5–8].

Abbreviations: ADL, activities of daily living; DQ, development quotient; ERT, enzyme replacement therapy; FIM, functional independence measure; GAG, glycosaminoglycan; HSCT, hematopoietic stem cell transplantation; IQ, intelligence quotient; JSPH, Japanese Society for Pediatric Hematology; MPS, mucopolysaccharidosis; MRI, magnetic resonance imaging; SD, standard deviation.

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Enzyme replacement therapy (ERT) for MPS II was approved in the USA and Europe in 2006, and in Japan in 2007. Its efficacy has been demonstrated for visceral organ and soft connective tissue involvement [9,10], but poor or no efficacy was observed for brain involvement [11,12] because of poor penetration across the blood–brain barrier. Poor efficacy has also been speculated towards hard connective tissues such as bone and heart valves because of poor vascularity. Moreover, weekly injection can prove inconvenient to patients and their families, and the high cost of treatment is another issue to be taken into consideration.

MPS II is the most frequent type of MPS in Asian patients, accounting for 60% of all MPS types in Japan. Before the approval of ERT, HSCT was indicated for MPS II as a standard therapy in Japan. The efficacy of HSCT on visceral organs was clear and similar to that of ERT [13]. However, efficacy on the brain or heart valves has not been clearly evaluated for either ERT or HSCT.

We present the results of a retrospective evaluation of the efficacy of HSCT on MPS II by collecting the clinical records of the patients with MPS II who received HSCT from 1990 to 2003. We also analyzed the answers to a questionnaire given to two cohorts: HSCT-treated and HSCT-untreated (natural history) MPS II patients.

2. Methods

2.1. MPS II classification

Disease severity was evaluated in all patients into four types (A–D) on the basis of chronological development, history of disease onset, initial symptoms, and clinical records before transplantation. Because of the wide spectrum of clinical phenotypes in MPS II, it is important to compare patients within the same type of disease for the evaluation of efficacy. Types A and B are attenuated forms with normal intelligence, while Types C and D are severe forms with mental impairment. MPS II was classified as follows:

- Type A is the most attenuated form. Onset is at school age with joint stiffness. Patients show normal intelligence, can go to and learn at a normal school, and work.
- Type B shows onset before school age with joint stiffness and/or abdominal distension. They show normal intelligence in primary school but hearing and physical impairments may impact development to a low degree in high school.
- Type C is a severe form. The abnormality is noted at ≥2 years of age. They start to speak words at 12–18 months of age and speak sentences at 2–3 years of age. Developmental delay and abnormal features become obvious after 3 years of age.
- Type D is a most severe form. The abnormality is noted at <2 years of age. Abnormal features are obvious around 1 year of age. Speech is definitely delayed. They start to speak words at ≥2 years of age (or may not speak), but sentences are never spoken.

2.2. Retrospective study from transplanted patients' records

This study was approved by the HSCT committee the Japanese Society of Pediatric Hematology (JSPH) and the ethics committees of the participating institutes.

A questionnaire was sent to 12 transplant centers in Japan to ask whether they had any type of MPS patients who had received HSCT and were surviving with donor cell engraftment and complete or incomplete chimera. We then mailed the physicians in charge of the patients with MPS II to obtain informed consent from the patients and/or their guardians so that data could be collected from their clinical records.

School status, movement and daily activities, conversation, and toileting were graded into Levels A (independent), B (assisted occasionally), C (assisted in every event), and D (bedridden, lack of communication, or wholly assisted) for each item from questionnaires and/or clinical records. Data on intelligence quotient (IQ) and development quotient (DQ) were also collected from clinical records. Functional independence measure (FIM) score was also analyzed and compared with the natural history of the disease as described in a previous report [14].

Brain magnetic resonance imaging (MRI) abnormalities were classified into four distinct types (Categories I–IV) and graded by scores according to a previous report [15]. The score was judged by two pediatricians and one radiologist. The categories were as follows:

- Category I. Cystic or cribriform lesions were graded from T1-weighted MRI as follows: 0 = none; 1 = mild (≤10 cystic lesions<3 mm); 2 = moderate (>10 small cystic lesions of<3 mm); and 3 = severe (many cystic lesions including those >3 mm).
- Category II. White matter signal changes observed on T2-weighted MRI were graded as follows: 0 = none; 1 = mild (a few limited to the periventricular area); and 2 = severe (in most parts of the periventricular area and other white matter areas).
- Category III. Ventricular enlargement was graded as follows: 0 = none; 1 = mild (<3 mm widening of the third ventricle without temporal horn dilatation); 2 = moderate, (>5–10 mm widening of the third ventricle); and 3 = severe (>10 mm dilatation of the third ventricle with bulbous configuration).
- Category IV. Brain atrophy was graded as follows: 0 = none, 1 = mild (mild widening of Sylvian and interhemispheric fissures by <3 mm, but not all of the sulci are involved); 2 = moderate (widening of all fissures and sulci by 3–5 mm); and 3 = severe (widening of all fissures and sulci by >5 mm with definite loss of cortex and white matter).

Cardiac valvular regurgitations were analyzed by color Doppler echocardiogram with each valve graded according to severity into four levels (I–IV) by the Sellers' classification [16].

Urinary glycosaminoglycan (GAG) was analyzed as the amount of uronic acid. These data were compared with the values in HSCT-untreated MPS II patients and also with those in ERT-treated MPS II patients.

2.3. Family questionnaire analysis

We sent a questionnaire to each of the 60 families with 66 MPS II patients registered with "the Japanese MPS Family Society". Information was collected about chronological development and course of deterioration for both HSCT-treated and HSCT-untreated (natural history) patients. Patients were first classified according to MPS II Types A–D on the basis of information on chronological development, before HSCT if performed, and at disease onset. Data were compared between HSCT-treated and HSCT-untreated patients for MPS II Type C or D patients.

3. Results

3.1. Retrospective study from transplanted patient records

Among transplanted patients with MPS, 63% (26/41) had MPS II. The 5-year survival rate after treatment of MPS II was 88.5% during the period from 1990 to 2003. Clinical records were collected for the 21 surviving patients (81%) from eight hospitals: Type A (n = 1), Type B (n = 6), Type C (n = 7), and Type D (n = 7) [Tables 1 and 2]. Donor state, transplantation protocol, and chimeric status are also summarized in Table 2. Two patients with Type B disease (patients 10-3 and 10-5) received total body irradiation (TBI) in the transplantation protocol. The donors for patients 10-7 and 7-6 were carrier siblings: patient 10-7 showed extremely low iduronate 2-sulfatase activity (25% of normal) even though complete chimera was obtained, while iduronate 2-sulfatase activity was normal in patient 7-6. Chimeric status was determined by short tandem repeats analysis in all patients except for four patients (patients 10-7, 7-8, 7-1, and 1-1) where sex chromosome was

Table 1
Patient numbers for each MPS II type and the results of HSCT effectiveness.

	No. of patients			
	Type A	Type B	Type C	Type D
Retrospective study from transplant patient records (n=21)	1	6	7	7
ADL (see Table 2)				
Patients analyzed (n=13)	1	3	5	4
Patients stabilized/improved from baseline	1	2	3	4
IQ/DQ (see Table 2)				
Patients analyzed (n=11)	0	2	4	5
Patients stabilized/improved from baseline	0	2	1	0
FIM (see Table 2)				
Patients analyzed (n=11)	1	1	6	3
Patients stabilized/improved from baseline	1	1	2	1
Brain MRI (see Tables 2 and 3)				
Patients analyzed (n=17)	1	6	5	5
Patients stabilized/improved from baseline (see Tables 2 and 3)	0	5	4	2
Cardiac valvular regurgitation (see Tables 2 and 4)				
Patients analyzed (n=21)	1	6	7	7
Patients stabilized/improved from baseline	1	4	5	6
Family questionnaire analysis (n=60)	7	13	26 (see Table 5)	14
HSCT (+) (n=17); [no. rejected]	3 [1]	3 [1]	7	4
HSCT (−) (n=43)	4	10	19	10

Abbreviations: ADL, activities of daily living; DQ, development quotient; FIM, functional independence measure; IQ, intelligence quotient; MRI, magnetic resonance imaging.

analyzed. The activity of iduronate 2-sulfatase in patient 1-3 showed the lower limit of normal activity, probably because of incomplete chimera. All other patients showed activity within the mean ± 1 SD of normal. Age at transplantation was 64.2 ± 30.2 months. The mean follow-up period was 115.7 ± 41.4 months. Patient numbers for each MPS II type and a brief summary of results for HSCT effectiveness are shown in Table 1.

Clinical background and outcome among HSCT-treated MPS II patients are detailed in Table 2. Not every patient underwent all clinical examinations. Answers to the questionnaire were obtained for the analysis of ADL (school status, movement and daily activities, conversation, and toileting) from 13 patients: Type A (n=1), Type B (n=3), Type C (n=5), and Type D (n=4). Two patients with attenuated forms of the disease (patients 1-3 and 7-3) maintained a normal level of ADL (Level A) for each item throughout the observation period. None of the patients with severe forms of the disease except two Type C patients (patients 5-1 and 1-1) showed deterioration from baseline status.

IQ/DQ data were available for 11 patients: Type B (n=2), Type C (n=4), and Type D (n=5). Two Type B patients (7-3 and 7-2) showed an IQ within the normal range both at baseline and at the most recent assessment. Deterioration was observed in two Type C patients (5-2 and 7-6) and two Type D patients (7-4 and 12-1). One Type C (patient 5-1) and one Type D (patient 8-2) showed such severe deterioration at baseline that evaluation of change was not possible. One Type C patient (7-1) and two Type D patients (7-5 and 9-1), whose IQ/DQ were >70 at baseline, maintained their developmental status without deterioration, while DQ decreased with increasing age (Table 2).

FIM score was available in 11 patients: Type A (n=1), Type B (n=1), Type C (n=6), and Type D (n=3). Patients with Type A/B disease maintained scores in the normal range. Three Type C/D patients (7-8, 7-1, and 4-1) showed disease attenuation in FIM score when compared with the natural history described in a previous report [14]. One Type C (patient 7-8) and one Type D (patient 4-1) showed disease attenuation in FIM score for motor function, while the score for cognition did not differ from untreated patients. One Type C (patient 7-1) showed disease attenuation in FIM scores for both motor function and cognition. Other patients with severe forms of the disease (4 Type C and 2 Type D) showed no difference as compared to the previously reported untreated patients [14]. The results are summarized in Table 2.

IQ/DQ and FIM scores were both obtained in seven patients: one Type B (patient 7-2), four Type C (patients 5-2, 7-6, 7-1, and 5-1), and two Type D (9-1 and 12-1). Among these patients with Type C/D disease and brain involvement, only one patient (7-1) showed disease attenuation in both FIM score and developmental status. The remaining three Type C patients showed no difference in FIM score as compared to natural history. While developmental status and ADL improved in patient 9-1, no efficacy in FIM score was shown as compared to natural history.

Brain MRI data were analyzed in 17 patients: Type A (n=1), Type B (n=6), Type C (n=5), and Type D (n=5) [Table 2]. Improvements in Categories I and III lesions were shown in nine (4 Type B, 2 Type C, and 3 Type D) and four patients (2 Type C and 2 Type D), respectively. Eight out of 17 patients (59%) had an improvement in total score. All of the six patients who showed an increase in total score had deterioration in Category IV lesions (brain atrophy). Three of these six patients had Type D disease (patients 7-4, 8-2, and 10-1). Two patients (7-1 and 4-1) who showed disease attenuation in FIM score also showed improvement in brain MRI abnormality scores. There was no difference in the effectiveness between the attenuated forms (Type A/B) and severe forms (Type C/D) of the disease or any correlation between the effectiveness of HSCT and age at HSCT, as summarized in Table 3.

Valvular regurgitation was analyzed for mitral, aortic, and tricuspid valves. Pulmonary valves showed insufficient lesions to warrant analysis. Twenty-one patients were analyzed: Type A (n=1), Type B (n=6), Type C (n=7), and Type D (n=7), i.e. a total of 63 valves. Results are summarized in Tables 2 and 4. Valvular regurgitation improved in 32% and stabilized in 56% of valves. There was no difference in efficacy between patients with the attenuated (Type A/B) and severe forms (Type C/D) of MPS II (data not shown). However, valvular regurgitation deteriorated more frequently in the patients transplanted at ≥6 years of age (5 valves out of 8 patients), as shown in Table 4.

The amount of urinary GAG was analyzed from urinary uronic acid concentrations. Mean urinary uronic acid concentrations in children ages 7–16 years were 18.0 ± 5.5 (n=24) and 165.5 ± 77.9 (n=9) mg/g creatinine for normal children and among untreated Types A–D MPS II patients, respectively. Urinary GAG in HSCT-treated MPS II patients was 24.8 ± 9.8 mg/g creatinine (n=7, ages 9–17 years). Urinary GAG in ERT-treated patients with MPS II at Osaka City University Hospital was 37.6 ± 14.3 mg/g creatinine (n=6, age 7–16 years).

3.2. Family questionnaire analysis

Answers to the questionnaire were collected for 60 patients with MPS II from 55 families. The numbers of HSCT-treated and HSCT-untreated patients were 17 and 43, respectively. As the questionnaire sheet was anonymous, we could not identify the patients analyzed in the clinical study described above. The patients were divided into Types A–D clinical forms (Table 1), as previously described. Six out of 20 Type A/B patients were treated by HSCT and two of them (one each with Types A and B) underwent rejection. Four of 14 Type D patients received HSCT. However, they showed deterioration before transplantation. We analyzed the efficacy of HSCT in 26 Type C patents with respect to disease progression by age at onset of speech deterioration, walking disability, and convulsion (Table 5). The numbers of patients in the HSCT-treated and HSCT-untreated cohorts were 7 and 19, respectively. Mean ages of these cohorts were 145.7 ± 67.8 and 142.7 ± 88.6 months, respectively.

Seven Type C patients underwent HSCT at a mean age of 65.9 ± 22.1 months (range, 44–111 months). Before HSCT treatment, the seven patients showed no difference in developmental milestones as compared to the 19 HSCT-untreated patients. At the time of survey, 12 out of 19 (63%) HSCT-untreated patients showed deterioration of

Table 2
Clinical background and outcome among HSCT-treated MPS II patients (n = 21).

Patient no.	Disease type	Age at HSCT	Donor	Protocol	Chimeric status	GVHD	Follow-up	ADL (pre/post), [n = 13]				IQ/DQ (developmental age)	
								School status	Movement and daily activities	Conversation	Toileting	Pre	Post
1-3	A	19 y 8 m	Unrelated BM	CY + BU + ATG	50	No	6 y 7 m	(A/A)	(A/A)	(A/A)	(A/A)	NA	
10-3	B	4 y 11 m	Unrelated CB	CY + TBI	100	No	7 y 1 m	NA	NA	NA	NA	NA	
7-3	B	5 y 5 m	Normal sibling	CY + BU + ATG	100	No	8 y 7 m	(A/A)	(B/A)	(A/A)	(A/A)	114 (normal)	102 (normal)
7-2	B	6 y 0 m	Normal sibling	BU + ATG	Mixed	No	10 y 11 m	NA	NA	NA	NA	99 (normal)	91 (normal)
8-1	B	9 y 5 m	Normal sibling	CY + BU	100	No	12 y 7 m	(E/E)	(E/E)	(B/B)	(E/E)	NA	
10-7	B	7 y 9 m	Carrier sibling	CY + BU + ATG	100	No	11 y 3 m	(A/A)	(A/A)	(A/A)	(A/A)	NA	
10-5	B	11 y 6 m	Unrelated BM	CY + TBI	90	Yes	6 y 6 m	(A/D)*	(B/B)	(A/A)	(A/A)	NA	
5-2	C	3 y 4 m	Normal sibling	CY + BU	100	No	7 y 4 m	(B/B)	(C/B)	(B/B)	(D/B)	53 (3 y 11 m)	NA
7-8	C	4 y 3 m	Unrelated BM	CY + BU + ATG	100	No	7 y 4 m	NA	NA	NA	NA	NA	
7-7	C	5 y 5 m	Unrelated CB	CY + BU + ATG	100	No	7 y 7 m	NA	NA	NA	NA	NA	
7-6	C	5 y 9 m	Carrier sibling	CY + BU + ATG	100	No	6 y 11 m	(B/B)	(C/C)	(D/C)	(D/B)	25 (1 y 8 m)	NA
7-1	C	7 y 0 m	Normal sibling	CY + BU	100	Yes	16 y 3 m	(B/B)	(B/A)	(B/A)	(E/E)	78 (5 y 6 m)	65 (9 y 6 m)
5-1	C	7 y 3 m	Normal sibling	CY + BU	100	No	10 y 5 m	(B/B)	(C/D)*	(C/C)	(B/C)*	NA	NA
1-1	C	9 y 4 m	Normal sibling	CY + BU	100	No	16 y	(C/D)*	(C/D)*	(C/D)*	(C/D)*	NA	
7-4	D	2 y 0 m	Unrelated BM	CY + BU + ATG	100	Yes	9 y 11 m	NA	NA	NA	NA	50 (1 y 0 m)	NA
7-5	D	2 y 2 m	Normal sibling	CY + BU + ATG	96	No	8 y 8 m	NA	NA	NA	NA	70 (1 y 6 m)	29 (2 y 2 m)
9-1	D	2 y 2 m	Unrelated BM	CY + BU + ATG	100	No	12 y	(E/B)	(C/A)	(B/A)	(C/A)	100 (2 y 2 m)	40 (5 y 6 m)
12-1	D	2 y 6 m	Normal sibling	CY + BU	100	No	8 y 3 m	(E/B)	(C/C)	(C/C)	(D/D)	66 (5 y 6 m)	30 (1 y 10 m)
8-2	D	2 y 9 m	Normal sibling	CY + BU	100	No	12 y 3 m	(D/B)	(D/D)	(D/D)	(D/D)	NA	NA
4-1	D	4 y 2 m	Unrelated BM	CY + BU + ATG	100	No	5 y 5 m	(B/B)	(A/A)	(C/B)	(D/B)	NA	
10-1	D	5 y 4 m	Normal sibling	CY + BU + ATG	100	No	7 y 8 m	NA	NA	NA	NA	NA	

Abbreviations: ADL, activities of daily living; ATG, antithymocyte globulin; BM, bone marrow; BU, busulfan; CB, cord blood; CY, cyclophosphamide; DQ, development quotient; FIM, functional independence measure; HSCT, hematopoietic stem cell transplantation; IQ, intelligence quotient; m, month; MPS, mucopolysaccharidosis; MRI, magnetic resonance imaging; NA, not available (not found, not examined, and/or not measurable); TBI, total body irradiation; y, year.

[a] Regression of level or score.