Original Article
Pediatric haploidentical hematopoietic stem cell transplantation followed by high-dose cyclophosphamide increased neutropenia risk

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Abstract: Allo-haploidentical hematopoietic stem cell transplantation (HSCT) is an effective curative treatment for various refractory blood diseases. To identify risk factors for neutropenia after pediatric HSCT, we retrospectively enrolled 25 pediatric patients with hematological malignancies who underwent human leukocyte antigen (HLA)-haploidentical HSCT with high-dose, post-transplant cyclophosphamide (PT/Cy) as prophylaxis. Primary diseases included 13 cases of acute lymphoblastic leukemia, seven of acute myeloid leukemia, two of non-Hodgkin's lymphoma, two of chronic myelogenous leukemia, and one of juvenile myelomonocytic leukemia. All donor and recipient pairs had at least one or two HLA mismatches. The 3-year overall survival, event-free survival, non-relapse mortality (NRM), relapse rates (RR), and neutropenia incidence rates were 28%, 28%, 40%, 32%, and 40%, respectively. HLA-haploidentical HSCT with PT/Cy prophylaxis against graft versus host disease (GVHD) for pediatric hematological malignancies was associated with a greater incidence of neutropenia, high NRM, and RR. Donor age and CD34$^+$ cell dosage were significantly associated with the incidence of neutropenia. Furthermore, donor age was identified as an independent risk factor. In conclusion, PT/Cy after HLA-haploidentical HSCT can effectively prevent GVHD with full donor chimerism, but with a high incidence of relapse and TRM after engraftment.

Keywords: Pediatric, haploidentical hematopoietic stem cell transplantation, neutropenia, cyclophosphamide, risk factor

Introduction

Allo-haploidentical hematopoietic stem cell transplantation (HSCT) is an effective treatment for various refractory blood diseases. However, more than half of patients are unable to receive treatment due to failure to find HLA-matching donors in a timely manner. Theoretically, everyone has at least one HLA half-matched related donor (haploid). The more incompatible the HLA loci, the worse the transplantation efficacy, which leads to limited haploidentical transplantation [1-4]. Cyclophosphamide (Cy) is a highly effective anti-cancer agent. Clinical trials have demonstrated that high-dose Cy after non-myeloablative HLA-HSCT was beneficial for early and stable implantation, while the incidence of acute and chronic graft-versus-host disease (GVHD) as well as the non-relapse mortality (NRM) rate in recipients were comparable to identical sibling transplantation [5, 6]. Neutropenia is a common complication after HSCT due to infection, septicemia and GVHD [7, 8]. The most important causes are myelotoxic potential and ganciclovir use, which is reportedly associated with an incidence rate of neutropenia of up to 40% [8, 9].

Therefore, the aim of this study is to identify risk factors for neutropenia, especially the relationship between allo-HSCT with PT/Cy and neutropenia, in pediatric hematological malignancies. Here, we retrospectively analyzed pediatric patients with refractory hematological malignancies who underwent HLA-haploidentical HSCT combined with PT/Cy.

Materials and methods

Patients

We retrospectively reviewed the medical records of 25 children (13 boys and 12 girls;

Table 1. Patient characteristics

Cases	Diagnosis	HLA matching	Preparative regimens	Stem cell origins	GVHD	Neutropenia	Survival Disease	Survival Time (mo)
1	ALL (CR2)	A, DR	FBAY	BM	HVG	F[b]	BMF	2.5
2	CML (PR)	B, CW	FBAY	BM	aII[a]	F	Infection	5
3[a]	ALL (PR)	A, B, C	FBCY	BM	aIV	N	Relapse	4.5
4	AML (NR)	A, B, C, DR, DQ	FBVY	BM	N	Y[c]	Infection	6
5	AML (CR2)	B, DR	FBAY	BM	N	Y	N	54
6	ALL (CR1)	B, CW	FBVY	BM	N	N	N	43
7	ALL (PR)	A, B, CW	FBAY	BM	aII	N	Relapse	2
8	ALL (CR)	A, B, DR	FBVY	BM	aI	Y	N	48
9	NHL4 (NR)	B, DR	FBVY	PBSC	C[d]	N	HC	5
10	NHL (CR2)	A, B, C, DR, DQ	FBCY	PBSC	C	Y	cGVHD	10
11	ALL (CR)	A, B, C, DR, DQ	FACY	BM+PBSC	N	Y	N	28
12	AML (PR)	A, B, C	FAC+TBI	BM	aI	N	Relapse	8
13	AML (CR2)	A, B, C, DR, DQ	FBTY	BM	aII	N	N	25
14	ALL (CR)	A, B, C, DR, DQ	FBTY	BM	N	N	N	24
15	AML (NR)	B, DR, DQ	FBTY	BM	aI	F	Relapse	14
16[a]	AML (NR)	A, C, DR, DQ	FBC	BM	aIV	F	Relapse	3.5
17[a]	ALL (NR)	B, C, DR, DQ	FBC+TBI	BM	aIII	Y	Relapse	3
18	CML-ALL	A, C, DR	FBC+TBI	BM+PBSC	c	N	Infection	6
19	AML (CR)	B, DR	FBTY	BM	aI	N	Infection	1.5
20	ALL (D[e], CR)	A, B, C, DR, DQ	FBTY	BM+PBSC	N	Y	Relapse	4
21	CML (CR)	A, C, DQ	FBVY	BM	aI	N	N	16
22	ALL (D[e], CR)	A, B, C, DR, DQ	TBI+FVC	BM+PBSC	N	Y	Infection	5
23	ALL (CR2)	A, B, C	TBI+FVC	BM+PBSC	aIII	Y	GVHD	6
24	JMML (PR)	A, C, DQ	FBVY	BM+PBSC	N	N	Relapse	3
25	ALL (CR1)	A, B, C, DR	TBI+FVC	BM+PBSC	N	Y	Infection	3

NHL: Non Hochkin's lymphoma, JMML: juvenile myelomonocytic leukemia, NR: none remission, aGVHD: acute graft-versus-host disease, cGVHD: chronic graft-versus-host disease, HVG: host-versus-graft, HC: hemorrhagic cystitis, FBAY: Flu+Bu+Ara-C+Cy, FBVY: Flu+Bu+VP-16+Cy, FBCY: Flu+Bu+CCNU+Cy, FACY: Flu+Ara-c+CCNU+Cy, FACT: Flu+Ara-c+Cy+TBI, FBTY: Flu+BU+TT+Cy, FBC: Flu+Bu+CCNU, FVC: Flu+ VP-16+Cy. [a]Donor lymphocyte infusion for relapse resulted in GVHD. [b]The neutropenia after day 28 cannot be evaluated. [c]Yes; [d]cGVHD; [e]Double expression.

median age, 9.0 years; age range, 1.2-13.0 years) with refractory hematological malignancies who underwent HLA-haploidentical HSCT in our center between June 2009 and November 2012. Patient demographics are described in **Table 1**. Of these patients, 13 were diagnosed with acute lymphoblastic leukemia (ALL), 7 with acute myeloid leukemia (AML), 2 with non-Hodgkin's lymphoma (NHL), 2 with chronic myelogenous leukemia (CML), and 1 with juvenile myelomonocytic leukemia (JMML). The pretransplant status of these patients is listed in **Table 1**. Of these patients, 17 underwent HSCT from a parent donor and eight from haploidentical sibling donors. The median donor age was 33 years (range, 4-46 years). There were two HLA-mismatches in six donor-recipient pairs and > 3 in 19 pairs.

Mobilization, collection, and infusion of hematopoietic stem cells

Stem cell sources included bone marrow (BM) in 16 cases, granulocyte colony-stimulating factor (G-CSF)-primed peripheral blood stem cells (PBSCs) in two, and BM+PBSCs in seven. All PBSCs and those collected from BM were primed with G-CSF at dose of 10 μg/kg body weight (BW)/day for 5 days. The recipients received a median of $1.6×10^8$ (range, 1.3-$16.1×10^8$) BM nuclear cells/kg BW for BM therapy, $9×10^8$ (range, 9-$9×10^8$) PBSCs/kg BW for PBSC therapy, or a median of $4.5×10^8$ (range, 0.18-$10.2×10^8$) nuclear cells/kg BW for BM+PBSC transplant therapy. The median number of total nucleated cells, mononuclear cells, and CD34^{+} cells were $8.3×10^8$ (range,
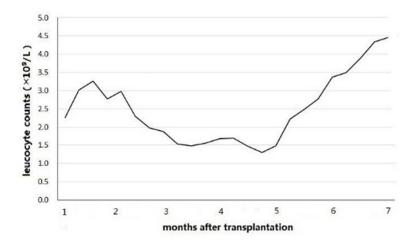
Figure 1. Average leucocyte counts of 10 haploid transplants. The curing showing ten recipients appeared to have Neutropenia 2 to 6 months after the transplantation.

3.6-16.8×10^8)/kg BW, 3.3×10^8 (range, 0.18-10.2×10^8)/kg BW, and 7.0×10^6 (range, 2.2-17.4×10^8)/kg BW, respectively.

Preparative regimen

Patients received intravenous Cy at a dose of 14.5-50 mg/kg BW/day on days-9 and -8, fludarabine i.v. at a dose of 40 mg/m^2/day from day-6 to day 2, and busulfan i.v. at a dose of 3.2 mg/m^2/day (or p.o. 4 mg/m^2/day) from day-7 to day-4. On day-3, six patients additionally received cytarabine i.v. at 4-6 g/m^2/day and nine received etoposide at 400-600 mg/m^2/day, while six received oral semustine at 250 mg/m^2/day. On day-1, six patients underwent total body irradiation (TBI) at a dose of 200 cGy. Patients undergoing pretreatment TBI received a subcutaneous injection of G-CSF at a dose of 3 µg/kg BW/day from post-transplantation day 4 until the absolute neutrophil count (ANC) increased to ≥ 0.5×10^9 cells/L plasma for 3 days.

GVHD and prevention of transplant rejection

After stem cell infusion, all 25 patients received Cy, tacrolimus, and mycophenolate mofetil (MMF) to prevent GVHD in addition to Cy on days +3 to +4. Among these patients, 10 received doses of 50 mg/kg BW/day, 15 received doses of 40 mg/kg BW/day and all were given the same mesna dose to prevent hemorrhagic cystitis. On day +5, patients started to receive tacrolimus i.v. at 0.03 mg/kg

BW/day, which was changed to oral administration after restoration of intestinal function. The dose was adjusted to ensure the blood trough level concentration was maintained at 5~15 µg/L. The dose was gradually decreased from 90 days post-transplantation and stopped at 180 days if GVHD did not occur. On day +5, MMF was orally administered at a dose of 15 mg/day three times a day, which was stopped on post-transplant day 35 if GVHD did not occur. Patients with acute GVHD (aGVHD) grade ≥ II were also given methylprednisolone at 1-2 mg/kg BW/day at relapse or rejection, as immunosuppression must be reduced or stopped in such cases. Donor lymphocyte infusion (DLI) or PBSC was infused in six cases, but the responses were insufficient. The initial DLI dose was 1×10^6/kg and infusion was repeated 2 weeks later. The dose was increased to 5×10^6/kg according to the engraftment test results, and each patient received an infusion no more than 3 times per donor.

Other therapies

Infusion of heparin sodium and ursodeoxycholic acid (p.o.) was used to prevent veno-occlusive disease. Ganciclovir (10 mg/kg BW/day) was administered to prevent cytomegalovirus (CMV) infection and itraconazole was administered on post-transplant day 5 to prevent fungal infections.

Donor engraftment analyses and definitions of neutropenia after post-transplant day 28

The myeloid engraftment time refers to the time from which the ANC was ≥ 0.5×10^9/L for 3 consecutive days. The platelet engraftment time refers to the time from which no platelet infusion was conducted, while the platelet count was ≥ 20×10^9/L for 7 consecutive days. We used polymerase chain reaction analysis of interspersed repeat sequences of the peripheral blood or BM short fragments, chromosome *in situ* hybridization, and ABO blood type to identify the chimeric status of the recipients. Cytopenia was defined as a white blood cell

Table 2. Univariate analysis for neutropenia

	Neutropenia	No neutropenia	P value
Patient's age			
> 6 years	8 (42.1)	11 (57.9)	
≤ 6 years	2 (33.3)	4 (66.7)	0.54
Donor's age			
> 40 years	6 (75)	2 (25)	
≤ 40 years	4 (23.5)	13 (76.5)	0.028
Recipients gender			
Male	3 (23.1)	10 (76.9)	
Female	7 (58.3)	5 (41.7)	0.082
Donors gender			
Male	6 (42.9)	8 (57.1)	
Female	4 (36.4)	7 (63.6)	0.53
Gender mismatch[a]			
Other	9 (50)	9 (50)	
Female into male	1 (14.3)	6 (85.7)	0.11
HLA mismatch			
2	1 (16.7)	5 (83.3)	
> 3	9 (47.4)	10 (52.6)	0.19
Stem cell source			
PBSC	1 (50.0)	1 (50.0)	
BMT	4 (25.0)	12 (75.0)	
PBSC+BMT	5 (71.4)	2 (28.6)	0.10
CD34+ cell dose			
> 6.4×10^6	2 (16.7)	10 (83.3)	
≤ 6.4×10^6	8 (61.5)	5 (38.5)	0.041
ABO mismatch			
Yes	7 (36.8)	12 (63.2)	
No	3 (50.0)	3 (50.0)	0.45
Donor's source			
Parent	6 (36.8)	11 (63.2)	
Sibling	4 (50.0)	4 (50.0)	0.45
Post-transplant Cy			
40 mg/kg	6 (40.0)	9 (60.0)	
50 mg/kg	4 (40.0)	6 (60.0)	0.65
aGVHD			
No	6 (54.5)	5 (45.5)	
I-II	2 (20.0)	8 (80.0)	
III-IV	2 (50.0)	2 (50.0)	0.24
CGVHD			
Yes	1 (33.3)	2 (66.7)	
No	9 (40.9)	13 (59.1)	0.65
CMV infection			
Yes	6 (50.0)	6 (50.0)	
No	4 (30.8)	9 (69.2)	0.28

[a]There is no different incidence rate of neutropenia between donor is female when the recipient is male and the others' sex mismatch.

count of < 3.0×10^9 cells/L, hemoglobin concentration of < 90 g/L, ANC of < 0.5×10^9/L, or platelet count of < 20×10^9/L, which indicated that the treatment regimen must be continued for more than 4 weeks. An ANC of < 0.5×10^9/L indicated a state of neutropenia, thus treatment was continued for more than 4 weeks. We used transfusion support after day 28 as a surrogate marker of anemia and thrombocytopenia. Since a state of anemia cannot be continued for 4 weeks, there was no available data regarding the incidence of anemia and thrombocytopenia.

Statistical analysis

Patient characteristics are summarized using medians and ranges for continuous variables. The incidence of neutropenia among different groups after day 28 was estimated using the chi-square test. A correlation model was used to assess associations between different groups and the frequency of neutropenia. A logistic regression model was used to estimate odds ratios and 95% confidence intervals for risk factors associated with neutropenia. Covariates included patient age, donor age, patient sex, donor sex, sex mismatch, HLA mismatch, stem cell source, CD34+ cell dose, ABO mismatch, donor source, post-transplant Cy dose, aGVHD, chronic GVHD (cGVHD), and CMV infection. Variables with a significance level of > 0.05 in the correlation model were candidates for the logistic regression model. All reported probability (*p*) values are two-sided.

Results

Implantation and chimerism

Sustained donor engraftment was demonstrated in all 25 patients within 28 days after transplantation, and the donor-chimerism rate was > 95%, while BM failure was observed in one patient 60 days later in whom hematopoietic function was not recovered. The median neutrophil engraftment time was 22.5 (range, 17-46) days and the median platelet engraftment time was 20.5 (range, 12-67) days. Among the 25 cases, neutrophil engraftment failed in one and platelet engraftment failed in three.

GVHD occurrence

Of the 25 cases included in this study, 11 (44%) developed GVHD (44%, 11/25), including seven

(28%) that were acute grade I-II and well controlled after receiving 1-2 mg of methylprednisolone/kg BW/day and one (4%) case developed aGVHD grade III-IV. Moreover, three cases (12%) developed aGVHD grade III-IV after DLI or PBSC infusion due to relapse, while three other cases (12%) developed cGVHD, of which one involved the skin, one the lung, and one the hematopoietic system, respectively.

Primary disease relapse and recipient survival

Follow-up was conducted until December 2013, with a median follow-up duration of 25 (range, 13-54) months. Eighteen of the 25 patients died, yielding a mortality rate of 72%, with median survival duration of 6 (range, 1.5-26) months. Eight patients died of primary disease recurrence, six mainly due to infection, one mainly due to refractory hemorrhagic cystitis, one mainly due to cGVHD, one mainly due to aGVHD, and one mainly due to BM failure after rejection. The 3-year overall survival (OS), 3-year event-free survival (EFS), NRM, RR, and neutropenia incidence rates were 28%, 28%, 40%, 32%, and 40% respectively. Of the patients who died, nine had ALL, five had AML, two had NHL, one had JMML, and two had CML. Of all 25 patients, 10 with non-remission of primary disease prior to transplantation died (**Table 1**).

Main complications

All 25 patients had varying degrees of secondary fever caused by infection, while 12 were positive for CMV DNA within 100 days of treatment. Although no patient died from CMV infection, as all became negative after ganciclovir treatment, two developed varicella-zoster virus infection, one died from viral encephalitis, two were confirmed with sepsis by blood culture results (one with *Klebsiella pneumoniae* and one with *Chryseobacteriummeningosepticum*), and fungal pneumonia was highly suspected in five. All of the six cases with hemorrhagic cystitis received Cy (50 mg/kg), although one patient died. There was no case of hepatic veno-occlusive disease, while 10 recipients appeared to develop severe cytopenia 2-6 months post-transplantation (**Figure 1**).

Effect factors for neutropenia after day 28

Among the 25 patients, neutropenia after day 28 was significantly associated with donor age and CD34$^+$ cell dose, but not Cy dosage (**Table 2**). The correlation model revealed that donor age was significantly positively correlated with neutropenia after day 28 ($r = 0.49$, $P = 0.013$), while the CD34$^+$ cell dose was significantly negatively correlated ($r = -0.45$, $P = 0.021$) (**Table 3**). The logistic regression model identified donor age (> 40 years) as a significant risk factor for neutropenia after day 28, but not CD34$^+$ cell dose (**Table 4**).

Discussion

HLA-haploidentical HSCT presents a viable treatment option for children with hematologic malignancies who are in urgent need of treatment after failing to find HLA-matching donors. However, compared with HLA-matched HSCT, HLA-haploidentical HSCT is associated with many complications, such as aGVHD, high engraft failure rate, slow hematopoietic reconstitution, delayed immune reconstitution, and a high incidence of fatal infections [10-13]. To address these issues, it was previously common to increase the infusion amount of CD34$^+$ cells, selectively inhibit donor T cell depletion (TCD) in vivo or in vitro, enhance pretreatment schemes, and increase the use of immunosuppressive agents, among other methods [14]. However, over-enhanced pretreatment schemes are likely to increase the incidence of transplant-related mortality (TRM) and organ toxicity, as excessive TCD is likely to cause transplant rejection, increase the risk for infection, delay hematopoietic recovery, or weaken graft-versus-leukemia effects, as well as increase the incidence of recurrence [15].

In this study, we administered HLA-haploidentical HSCT with PT/Cy and all patients achieved complete engraftment with early stage blood recovery. In one case, the hemogram after engraftment was quickly reduced to below the clinical standard criteria for engraftment, while BM or peripheral cells were fully derived from the donor. Two months later, the patient received a high-dose PBSC infusion, but hematopoietic function was not recovered. We suspected that this might have been associated with a microenvironment of BM injury. Despite the great disparity in the HLA-haploidentical related recipients, the incidence of aGVHD and extensive cGVHD were quite similar to previous reports of regimens using

Table 3. Correlation factors of neutropenia after day 28

	Neutropenia after day 28		
	N	r	*P* value
Patient's age			
> 6 years	8		
≤ 6 years	2	0.076	0.71
Donor's age			
> 40 years	6		
≤ 40 years	4	0.49	0.013
Recipients gender			
Male	3		
Female	7	-0.36	0.078
Donors gender			
Male	6		
Female	4	0.066	0.75
Gender mismatch[a]			
Other	9		
Female into male	1	0.32	0.11
HLA mismatch			
2	1		
> 3	9	-0.26	0.19
Stem cell source			
PBSC	1		
BMT	4		
PBSC+BMT	5	-0.31	0.12
CD34+ cell dose			
> 6.4×10^6	2		
≤ 6.4×10^6	8	-0.45	0.021
ABO mismatch			
Yes	7		
No	3	-0.11	0.58
Donor's source			
Parent	6		
Sibling	4	-0.14	0.50
Post-transplant Cy			
40 mg/kg	6		
50 mg/kg	4	0	1
aGVHD			
No	6		
I-II	2		
III-IV	2	0	1
CGVHD			
Yes	1		
No	9	-0.05	0.81
CMV infection			
Yes	6		
No	4	-0.19	0.34

r correlation coefficient. [a]There is no correlation about neutropenia between donor is female when the recipient is male and the others' sex mismatch.

HLA-matched related and unrelated donors [16]. Among the cases included in this study, the incidence of GVHD was not especially high and most instances of aGVHD were grades I–II. These results suggest that the protocol effectively promoted hematopoietic recovery and prevented GVHD. The results of many pre-clinical studies suggested that Cy administration after receiving solid organ transplantation or HSCT had a stronger inhibitory effect on graft versus host reaction compared with application prior to transplantation [17]. However, another study reported that low-dose Cy was not as effective as CsA in preventing GVHD following HLA-matched sibling transplantation, and animal experiments revealed that a single administration of Cy (> 150 mg/kg BW) within 48 to 72 h after transplantation induced resistance among minor histocompatibility antigens [18]. Other studies have found fewer CD4+ Foxp3+ cells in patients with aGVHD grade II-IV than with grades I-II, while interferon-γ RNA expression was greater in the former group, indicating that aGVHD was highly associated with regulatory T cell depletion [19]. Our results also supported the hypothesis that PT/Cy is important to the killing of T cell clones, which are involved in the development of GVHD [20]. Preliminary studies with transgenic mice in which Foxp3+ Tregs were selectively depleted suggested that this absence abrogates the protection provided by PT/Cy against GVHD [21]. Meanwhile, Cy had no effect on the restoration of lymphocytes, including regulatory T lymphocytes. The main mechanism of GVHD prevention with PT/Cy was the high aldehyde hydrogenase expression and relatively static properties of stem cells, which are apparently not affected by high-dose Cy [22]. Conversely, the activated T lymphocytes were selectively removed by Cy administration, leading to an effective reduction in the occurrence of GVHD [23]. This conclusion is consistent with recent observations that Foxp3+ Tregs are critical to tolerance induction in major histocompatibility complex-matched and -mismatched models using anti-T cell antibodies and co-stimulatory blockade [24]. Luznik et al. applied Cy (50 mg/kg BW/day) for 3-4 days post-transplantation to prevent GVHD, which was based on animal experiments that reported that CsA may hinder Cy-induced immune tolerance, while tacrolimus and MMF may be applied after administration of the last dose of Cy [23].

Table 4. Risk factor for neutropenia after day 28

	N	HR (95% CI)	*P* value
Donor's age			
> 40 years	6	1	
≤ 40 years	4	9.75 (1.38-68.78)	0.022

HR: hazard ratio, CI: confidence interval.

One study reported that among 68 patients with hematologic malignances undergoing non-myeloablative HLA-haploidentical HSCT combined with Cy, the incidence of aGVHD grades II-IV and III-IV at postoperative day 200 was 34% and 6%, respectively [5], while the incidence of aGVHD grades II-IV and III-IV 100 days after myeloablative sibling HSCT was 43% and 10%, respectively [15]. The 1-year cumulative non-myeloablative HLA-haploidentical HSCT and NRM rates for the former were 51% and 15%, respectively, and the 2-year OS and EFS rates were 36% and 26%, respectively [5], indicating that the non-myeloablative HLA-haploidentical HSCT rate was a major problem. In this study, the mortality rate was relatively high, mainly due to non-myeloablative HLA-haploidentical HSCT and infection. Most patients who underwent non-myeloablative HLA-haploidentical HSCT had ALL with a high-risk for non-remission of bone marrow morphology prior to transplantation, indicating that disease status prior to transplantation might significantly influence prognosis. Infection was a major cause of NRM after transplantation. We suspected that this was mainly because neutropenia was likely to cause infection and treatment was discontinued due to medical costs. We found that in 22 appreciable cases, 10 developed neutropenia 2 to 6 months after transplantation. Other studies reported that the incidence of neutropenia might be associated with BM GVHD, CMV infection, immunosuppressant dose, pre-treatment intensity, HLA matching, as well as CD34$^+$ cell numbers, among other factors [25, 26]. Nakamae et al. indicated that patient age, CMV seropositivity, unrelated donor status, HLA mismatched donor, MMF use, and lower CD34$^+$ cell dose (≤ 6.4×10^6/kg BW) were significant risk factors for the development of cytopenias, especially neutropenia after transplantation [27]. Non-myeloablative conditioning was associated with significantly reduced incidences of anemia and thrombocytopenia, but not neutropenia. In the present study, the incidence of neutropenia was higher than that reported by Nakamae et al [27]. Our analysis showed that low CD34$^+$ cell dose and especially advanced donor age were associated with a greater incidence of neutropenia. We hypothesized that in pediatric transplantation, older donors may have a greater frequency of natural factors associated with neutropenia, such as functional deterioration of cells, greater risk of infections, and low immune function, etc. Surprisingly, CD34$^+$ cell dose (≤ 6.4×10^6/kg BW) was not identified as a risk factor, which may be due to the relatively limited number of included cases. Also, other data showed that CMV infection was correlated to delayed platelet recovery after BM transplant [28]. However, in the present study, there was no correlation between CMV infection and neutropenia. Dominietto et al. analyzed platelet recovery in 342 patients after allogeneic HSCT and found that the severity of GVHD was associated with a greater decrease in platelet counts and reduced OS [29]. In the present study, no patient developed severe GVHD, which may be attributable to the high PT/Cy dose. Moreover, stem cell expression of aldehyde dehydrogenase might be influenced by ethnic and individual differences, leading to genetic polymorphisms regarding the efficacy of Cy, which might thereby impact hematopoietic recovery to various degrees. In our study, we found no correlation between the effect of two PT/Cy regimens (40 and 50 mg/kg BW) on the prevention of neutropenia and the incidence of GVHD. However, because of the limited number of cases, larger studies are needed in the future and the efficacy of individualized treatment regimens, such as Cy infusion and HSCT, must be further improved.

The results of this study suggest that PT/Cy after HLA-haploidentical HSCT can effectively prevent GVHD with full donor chimerism. However, there was a high incidence of relapse and TRM after engraftment. Finally, the study identified potentially modifiable factors that could be used before transplantation to minimize the risk of neutropenia, such as the inclusion of male patients and the use of higher doses of CD34$^+$ cell infusions. Revised protocols are needed in further prospective randomized studies to improve the efficacy of haploidentical HSCT in pediatric hematological malignancies.

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Disclosure of conflict of interest

None.

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References

[1] Askar M, Sobecks R, Morishima Y, Kawase T, Nowacki A, Makishima H, Maciejewski J. Predictions in the face of clinical reality: HistoCheck versus high-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease. Biol Blood Marrow Transplant 2011; 17: 1409.

[2] Kanda Y, Chiba S, Hirai H, Sakamaki H, Iseki T, Kodera Y, Karasuno T, Okamoto S, Hirabayashi N, Iwato K, Maruta A, Fujimori Y, Furukawa T, Mineishi S, Matsuo K, Hamajima N, Imamura M. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991-2000). Blood 2003; 102: 1541.

[3] Kawase T, Morishima Y, Matsuo K, Kashiwase K, Inoko H, Saji H, Kato S, Juji T, Kodera Y, Sasazuki T; Japan Marrow Donor Program. High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism. Blood 2007; 110: 2235.

[4] Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, Yamamoto K, Maruya E, Akatsuka Y, Onizuka M, Sakamaki H, Sao H, Ogawa S, Kato S, Juji T, Sasazuki T, Kodera Y; Japan Marrow Donor Program. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. Biol Blood Marrow Transplant 2007; 13: 315.

[5] Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, Gooley TA, Piantadosi S, Kaup M, Ambinder RF, Huff CA, Matsui W, Bolaños-Meade J, Borrello I, Powell JD, Harrington E, Warnock S, Flowers M, Brodsky RA, Sandmaier BM, Storb RF, Jones RJ, Fuchs EJ. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant 2008; 14: 641.

[6] O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, Rhubart P, Cowan K, Piantados S, Fuchs EJ. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant 2002; 8: 377.

[7] Bruno B, Gooley T, Sullivan KM, Davis C, Bensinger WI, Storb R, Nash RA. Secondary failure of platelet recovery after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2001; 7: 154.

[8] Salzberger B, Bowden RA, Hackman RC, Davis C, Boeckh M. Neutropenia in allogeneic marrow transplant recipients receiving ganciclovir for prevention of cytomegalovirus disease: risk factors and outcome. Blood 1997; 90: 2502.

[9] Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood 1996; 88: 4063.

[10] Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 27: 893.

[11] Szydlo R, Goldman JM, Klein JP, Gale RP, Ash RC, Bach FH, Bradley BA, Casper JT, Flomenberg N, Gajewski JL, Gluckman E, Henslee-Downey PJ, Hows JM, Jacobsen N, Kolb HJ, Lowenberg B, Masaoka T, Rowlings PA, Sondel PM, van Bekkum DW, van Rood JJ, Vowels MR, Zhang MJ, Horowitz MM. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol 1997; 15: 1767.

[12] Woolfrey AE, Gooley TA, Sievers EL, Milner LA, Andrews RG, Walters M, Hoffmeister P, Hansen JA, Anasetti C, Bryant E, Appelbaum FR, Sanders JE. Bone marrow transplantation for children less than 2 years of age with acute myelogenous leukemia or myelodysplastic syndrome. Blood 1998; 92: 3546.

[13] Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, Sanders JE, Stewart P, Buckner CD, Storb R, et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 1985; 313: 765.

[14] Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Cunningham-Rundles S, Dupont B, Hodes MZ, Good RA, O'Reilly RJ. Transplantation for severe combined immunodeficiency with HLA-A,B,D,DR incompatible parental marrow cells fractionated by soybean agglutinin and sheep red blood cells. Blood 1983; 61: 341.

[15] Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood 2001; 98: 3192.

[16] Burroughs LM, O'Donnell PV, Sandmaier BM, Storer BE, Luznik L, Symons HJ, Jones RJ, Ambinder RF, Maris MB, Blume KG, Niederwieser DW, Bruno B, Maziarz RT, Pulsipher MA, Petersen FB, Storb R, Fuchs EJ, Maloney DG. Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following nonmyeloablative conditioning for relapsed or refractory Hodgkin lymphoma. Biol Blood Marrow Transplant 2008; 14: 1279.

[17] Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. Biol Blood Marrow Transplant 2002; 8: 131.

[18] Mayumi H, Himeno K, Tokuda N, Nomoto K. Drug-induced tolerance to allografts in mice. VII. Optimal protocol and mechanism of cyclophosphamide-induced tolerance in an H-2 haplotype-identical strain combination. Transplant Proc 1986; 18: 363.

[19] Ash RC, Horowitz MM, Gale RP, van Bekkum DW, Casper JT, Gordon-Smith EC, Henslee PJ, Kolb HJ, Lowenberg B, Masaoka T, et al. Bone marrow transplantation from related donors other than HLA-identical siblings: effect of T cell depletion. Bone Marrow Transplant 1991: 7: 443.

[20] Eto M, Mayumi H, Tomita Y, Yoshikai Y, Nishimura Y, Nomoto K. Sequential mechanisms of cyclophosphamide-induced skin allograft tolerance including the intrathymic clonal deletion followed by late breakdown of the clonal deletion. J Immunol 1990; 145: 1303.

[21] Fujioka T, Tamaki H, Ikegame K, Yoshihara S, Taniguchi K, Kaida K, Kato R, Inoue T, Nakata J, Ishii S, Soma T, Okada M, Ogawa H. Frequency of CD4(+)FOXP3(+) regulatory T-cells at early stages after HLA-mismatched allogeneic hematopoietic SCT predicts the incidence of acute GVHD. Bone Marrow Transplant 2013; 48: 859.

[22] Kastan MB, Schlaffer E, Russo JE, Colvin OM, Civin CI, Hilton J. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. Blood 1990; 75: 1947.

[23] Luznik L, Bolanos-Meade J, Zahurak M, Chen AR, Smith BD, Brodsky R, Huff CA, Borrello I, Matsui W, Powell JD, Kasamon Y, Goodman SN, Hess A, Levitsky HI, Ambinder RF, Jones RJ, Fuchs EJ. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood 2010; 115: 3224.

[24] Kendal AR, Chen Y, Regateiro FS, Ma J, Adams E, Cobbold SP, Hori S, Waldmann H. Sustained suppression by Foxp3^{+} regulatory T cells is vital for infectious transplantation tolerance. J Exp Med 2011; 208: 2043.

[25] Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S, Felicini R, Falcinelli F, Velardi A, Ruggeri L, Aloisi T, Saab JP, Santucci A, Perruccio K, Martelli MP, Mecucci C, Reisner Y, Martelli MF. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol 2005; 23: 3447.

[26] Wingard JR, Chen DY, Burns WH, Fuller DJ, Braine HG, Yeager AM, Kaiser H, Burke PJ, Graham ML, Santos GW, et al. Cytomegalovirus infection after autologous bone marrow transplantation with comparison to infection after allogeneic bone marrow transplantation. Blood 1988; 71: 1432.

[27] Nakamae H, Storer B, Sandmaier BM, Maloney DG, Davis C, Corey L, Storb R, Boeckh M. Cytopenias after day 28 in allogeneic hematopoietic cell transplantation: impact of recipient/donor factors, transplant conditions and myelotoxic drugs. Haematologica 2011; 96: 1838.

[28] Verdonck LF, de Gast GC, van Heugten HG, Nieuwenhuis HK, Dekker AW. Cytomegalovirus infection causes delayed platelet recovery after bone marrow transplantation. Blood 1991; 78: 844.

[29] Dominietto A, Raiola AM, van Lint MT, Lamparelli T, Gualandi F, Berisso G, Bregante S, Frassoni F, Casarino L, Verdiani S, Bacigalupo A. Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. Br J Haematol 2001; 112: 219.