Haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for treatment of hematological malignancies in children

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ABSTRACT
To investigate the efficacy and safety of haploidentical (from family member donors) hematopoietic stem cell transplantation (HSCT) for children. 42 children under 14 yrs old with hematological malignancies underwent haploidentical HSCT. Outcomes were analyzed. Thirty-three children were classified as high-risk candidates. Of 42 patient/donor pairs, 4 (9.5%) were mismatched in 2 HLA loci, 15 (35.7%) in 3 loci, and 23 (54.8%) in 4 loci. Follow-ups were performed for a median of 1110 (449-1959) days after transplantation. All patients achieved stable engraftments. The cumulative incidence of acute graft-versus-host disease (aGVHD) of grade 2-4 was 57.2%, and that of grade 3-4 was 13.8%. The cumulative incidence of chronic graft-versus-host disease (cGVHD) was 56.7% for total and 29.5% for extensive. Twenty-seven patients survived with a 3-yr probability of leukemia-free survival (LFS), 57.3±8%, 18 of them were in the high-risk group. Fifteen patients died, 4 from infection, 7 from relapse of leukemia, 2 from heart failure, one from severe aGVHD, and one from lymphoproliferative disorders. The results encourage extending haploidentical HSCT without T cell depletion treatments to children with an indication for transplantation.

KEY WORDS
hematopoietic stem cell transplantation • haploidentical • hematological malignancies • children

INTRODUCTION
Despite recent advances in the diagnosis and treatment of childhood hematological malignancies, there are several subgroups of children that are at high risk of failing current chemotherapy regimens and require allogeneic hematopoietic stem cell transplantation (HSCT) to cure their disease [1,2]. The first choice of a donor for allogeneic HSCT is an HLA-identical sibling. However, this is not possible for patients who have no siblings. The alternatives are an unrelated HLA-matched donor, umbilical cord blood, or a family member that serves as an HLA haploidentical donor. The unsuccessful or time-consuming donor search process limits the application of unrelated donors for HSCT. Although cord blood transplantation (CBT) is more successful for children than for adults, its outcome is limited by incomplete hematopoietic and immune reconstitution [3,4]. Recently, we reported a novel method for haploidentical/mismatched HSCT from family members without in vitro T cell depletion, which showed survival, relapse, and graft-versus-host disease (GVHD) outcomes comparable to those of transplantation from identical siblings [5,6]. In this study, we report the results of 42 children under 14 yrs old that received HSCT from haploidentical family donors within 5 consecutive yrs. The toxicity and efficacy of this transplantation method for children were investigated.

MATERIALS AND METHODS
Patient Eligibility
Forty-two children under 14 (3-14) yrs old with hematological disorders underwent haploidentical HSCT between January 2002 and April 2006 at
a median of 762 (330-2738) days after diagnosis. The short-term results of 22 patients were reported in 2006 and subsequent progress was followed up in this study. None of the patients had HLA-identical related or unrelated donors, or a source of stem cells from umbilical cord blood. Twenty-four patients had acute lymphoblastic leukemia (ALL), twelve patients had acute myeloid leukemia (AML), and 6 patients had chronic myelogenous leukemia (CML) before HSCT. Twenty-nine children with acute leukemia presented with clinical and biological features that indicated a very high risk of relapse with conventional chemotherapy. Patients with CML were enrolled because allogeneic HSCT was the first-line therapy recommended in China for CML patients who were under 20 yrs old. Two patients with AML and 5 patients with ALL in the first and second remissions (CR1 and CR2, respectively) were enrolled based on fervent requests from their parents. All the children were treated with one protocol as described below. The Institutional Review Board of Peking University approved this study, and all patients’ guardians and donors signed consent forms for transplantation and stem cell collection.

Donor and Stem Cell Harvesting

For eligibility, HSCT recipients had to have allele types HLA-A, HLA-B, HLA-C, or HLA-DRB1. Low-resolution DNA techniques were used for HLA typing of the A and B loci. High-resolution techniques were used to define class II antigens. Donors were primed with granulocyte-colony stimulating factor (G-CSF, Filgrastim, Kirin, Japan; 5 μg/kg per day) injected subcutaneously for 5 consecutive days. The target mononuclear cell count (MNC) was 4-6×10^8 cells/kg recipient weight. On the fourth day, bone marrow cells were harvested. On the fifth day, peripheral blood stem cells (PBSC) were collected with a COBE Blood Cell Separator (Spectra LRS, COBE BCT, Inc., Lakewood, Colorado, USA) from a total blood volume of 10L. Forty patients received G-CSF-primed bone marrow combined with PBSC at transplantation; one patient received the G-CSF-primed bone marrow; and the other patient received PBSC. Surface markers of the cells in the grafts were determined by 2- or 3-color staining using monoclonal antibodies specific for CD34+, CD3+, CD4+, and CD8+ cells, essentially as described by Huang et al [7].

Conditioning Regimen and GVHD Prophylaxis and Therapy

Recipient patients underwent conditioning for 10 days before transplantation; the day of transplantation was designated day 0. The conditioning regimen consisted of arabinoside (Ara-C, 4 g/m²/d, i.v.) given on days –10 and –9; busulfan (Bu, 12 mg/kg, p.o. in 12 doses) given on days –8, –7, and –6; cyclophosphamide (Cy, 1.8 g/m²/d, i.v.) given on days –5 and –4; Simustine (Me-CCNU, 250 mg/kg, i.v.) given on day –3; and antithymocyte globulin (ATG, 2.5 mg/kg/d of the Sangstat product, i.v.) given on days –5 through –2. All transplant recipients received cyclosporine A (CsA), mycophenolate mofetil (MMF), and short-term methotrexate (MTX) for GVHD prophylaxis.

The diagnosis and grading of GVHD was done according to published criteria [8]. Supportive care was given as described previously [6]. DNA fingerprinting of short tandem repeats was used for confirmation of engraftment and determination of chimerism. Chromosomal fluorescent in situ hybridization (FISH) was also used to detect chimerism in patients with a donor of a mismatched gender. Acute GVHD (aGVHD) of grade 2 or higher was treated with methylprednisolone (0.5-1 mg/kg per day). GVHD manifested on the skin was treated with MTX [9]. When there was inadequate or no response to primary therapy, tacrolimus replaced cyclosporine A or anti-Tac monoclonal antibody (Daclizumab; Roche, Basel, Switzerland) was administered at 1mg/kg i.v. on days 1, 4, 8, and then at 7-day intervals for a total of 5 to 6 doses. The first-line therapy for chronic GVHD (cGVHD) was cyclosporine A combined with prednisone (0.5-1 mg/kg per day). Tacrolimus and azathioprine were used in refractory cases. Penicillamine was used in combination with medications listed above for patients with generalized skin involvement.

Definition

The severity of acute and chronic GVHD were diagnosed with the standard criteria [8]. The date of neutrophil recovery was defined as the first day of 3 consecutive days that an absolute neutrophil count of 0.5×10^9/L was achieved. The date of platelet engraftment was defined as the first day of 7 continuous days that an absolute platelet count of 20×10^9/L was achieved without the support of transfusion. At day +30, the disease response and chimerism were assessed in peripheral blood and bone marrow. Relapse was defined as a hematological recurrence of the primary disease. Death from leukemia was defined as death with refractory disease after transplantation, or as death from any cause following relapse after transplantation. Transplantation-related mortality (TRM) was defined as death during continuous post-transplantation remission.

Patients were stratified into standard-risk and high-risk groups according to their remission state before transplantation. High-risk candidates for haploidentical HSCT included patients with acute leukemia (AL) in CR1 and in CR2 with a cytogenetic marker of “poor-risk”, such as the Philadelphia chromosome; those in complete remission after CR 1; those in non-remission or in a relapse state before transplantation; and those with CML beyond the first chronic phase.
Supportive Care and Follow-Up

HSCT recipients were attended in isolation rooms with laminar airflow systems. To prevent infections of pneumocystis carinii, herpes simplex and varicella-zoster virus (VZV), and Candida albicans, patients were given prophylactic doses of trimethoprim/sulfamethoxazole (1-2g/d, p.o., from day –10 to day +30, and 2 days/wk up to day 90), acyclovir (0.4-0.8g/d, p.o. from day +1 to 1 yr), and fluconazole (50-100mg/d, p.o. from day –10 to myeloid recovery), respectively. To prevent cytomegalovirus (CMV) reactivation, patients were given ganciclovir (5mg/kg, i.v. bid.) from day –9 to –2. For treatment of pneumocystis carinii, trimethoprim/sulfamethoxazole was administered 4-6g/d, p.o. for 2-3 wks. Children with a history of fungal infection were given itraconazole for prophylaxis from day –10 up to myeloid recovery. For treatment of empiric and proven invasive fungal infections, itraconazole, caspofungin, or emfotericin B / Amphotec were given. Antifungal agents were used for 2-3 wks for empiric therapy of fever and for 3-4 mos for pneumonia with computer tomography (CT) findings of infiltration and other proven infections. CMV-DNA levels were determined twice a week before day +90; after day +90, in case of extensive cGVHD, blood was examined for pp65 antigenemia with PCR. When viremia was detected twice, preemptive therapy was started with ganciclovir, which was used for 4 to 6 wks since negative detection of antigenemia after the therapy. Refractory CMV antigenemia that was continuously positive with preemptive therapy, and CMV disease were treated with forscarnet (90-120mg/d, i.v.) and immunoglobulin (40mg/kg×7 days, i.v.) combined with ganciclovir. The course of therapy for proven CMV disease was 6-8 wks. All children received piperacillin alone or combined with amikacin for prophylaxis of bacterial infections from day –10 to myeloid recovery. The first line antibiotics for empiric therapy of fever during neutropenia were cefepime, meropenem, or imipenem and sodium cilastatin. If fever was not controlled and microbiological cultures were negative after 3-5 days of the first line therapy, vancomycin or teicoplanin was added.

Blood products were infused into patients that had hematocrit levels <25% and/or platelet counts <20 × 10^9/L. All blood products were irradiated prior to use.

During hospitalization, clinical status, adverse events, hematological parameters, and clinical biochemical parameters were monitored daily. After discharge from the hospital, patients were examined in the outpatient clinic once to twice per wk up to day 100 and at gradually longer intervals thereafter. Toxicities were graded according to WHO criteria.

Statistics

The primary endpoint of the study was the overall survival (OS) rate at 3 yrs from transplantation. Secondary endpoints included leukemia-free survival (LFS, defined as survival in continuous complete remission after transplantation), non-relapse mortality, incidence of relapse, and incidence and severity of acute and chronic GVHD. The day of stem cell transfusion was counted as day 0, and all intervals were calculated based on this date.

Patients were evaluated for aGVHD after engraftment, and for cGVHD starting 100 days after HSCT. Time to acute or chronic GVHD, relapse, survival, and LFS were measured from the date of HSCT (day 0). The risk factors calculated for univariate analysis included: age and gender of patients and donors; relation of the patients and donors; time from diagnosis to transplantation; HLA disparity; diagnosis and remission status before transplantation; cell subsets infused in the graft; time of myeloid and platelet engraftment; and the incidence of aGVHD, cGVHD, and infections. The Cox-regression model was not built due to the limited number of cases. The date of the last follow-up for all surviving patients was November 1, 2007. All reported P values were based on two-sided hypothesis tests. Alpha was set at 0.05. Numeric variables were analyzed as categories considering their value below or above the median of the entire cohort, as indicated in the Results section. Acute and chronic GVHD were analyzed as time-dependent variables. OS and LFS were estimated using the Kaplan-Meier method. The SPSS-13.0 software package was used for data analysis.

RESULTS

Characteristics of Patients and Engraftment

Twenty-eight donors were mothers and 12 donors were fathers of the patients. The other 2 donors were an uncle and a brother; both were haploidentical according to the familial spectrum of genetics analysis. All patients were mismatched at the allele level for HLA-A, HLA-B, HLA-C, and HLA-DRB1. Twenty-three (54.8%) patients were mismatched in 4 loci, 15 (35.7%) patients were mismatched in 3 loci, and 4 (9.5%) in 2 loci. The median age of the donors was 36 (17-43) yrs old. The patients were followed up until November 1, 2007 with a median of 1110 (449-1959) days after transplantation. According to the risk group definitions mentioned above, 2 patients with AML, 5 patients with ALL, and 2 patients with CML were stratified into a standard-risk group and 10 AML, 19 ALL, and 4 CML were stratified into a high-risk group. The characteristics of patients and donors are described in Table 1.

The median numbers of MNC infused at transplantation were 8.4 (1.1-15.4) ×10^8/kg. The cell
Median time from diagnosis (range) 289 (77-1825) days

Age at transplantation (yrs)
>1-5 3 7.1
>5-10 12 28.6
>10≤14 27 64.3

Disease and status Total/high-risk %
AML 12 28.7
CR1/CR2 7/5
≥CR3 4/4
Non-remission 1/1

ALL 24 57.4
CR1/CR2 15/10
≥CR3 5/5
Non-remission 3/3
Ph+* 1/1

CML 6 14.9
CIPI 2/0
AP 3/3
BC 1/1

Median time from diagnosis to transplantation (range) 289 (77-1825) days

Year of transplantation
2002-2003 13 30.9
2004 18 42.9
2005-2006.02 11 26.2

Donor-recipient sex match
- Female-male 15 35.7
- Female- female 13 31.0
- male-male 9 21.4
- male- female 5 11.9

Donor-recipient relationship
- Mother-child 28 66.7
- Father-child 12 28.5
- Uncle-nephew 1 2.4
- Siblings 1 2.4

Blood types of donor to recipient
- Matched 26 61.9
- Major mismatched 4 9.5
- Minor mismatched 11 26.2
- Major and minor 1 2.4

MNC indicates mononuclear cells; BM, bone marrow; PB, peripheral blood.

Subsets infused are listed in Table 2. All patients achieved stable engraftment and whole donor chimerism as detected by DNA fingerprinting of short tandem repeat and chromosomal FISH. The median times of myeloid and platelet recovery were 14 (9-22) days and 22 (8-90) days after transplantation, respectively. There was no significant association between the extent of HLA disparity and the time of myeloid or platelet recovery (Table 3).

**Graft-Versus-Host Disease and Opportunistic Infections**

Thirty-one patients developed aGVHD, with grade 1 in 11 patients, grade 2 in 16 patients, and grade 3 in 4 patients. Acute GVHD grade 2 occurred in 2 of 4 patients with HLA mismatched in 2 loci, 4 of 15 patients mismatched in 3 loci and 10 of 23 patients mismatched in 4 loci. Acute GVHD grade 4 was diagnosed in 2 patients with HLA mismatched in 3 loci, and in 2 patients mismatched in 4 loci, at days 13, 21, 21, and 29 after transplantation, respectively. Adverse outcomes were found to be unassociated with the extent of mismatched HLA-loci. The cumulative incidence of aGVHD was compared among different HLA disparities as shown in Figures 1a and 1b. The cumulative incidence of aGVHD grade 2-4 was 57.2% (Figure 1a), and that of aGVHD grade 3-4 was 13.8% (Figure 1b). The target organs of aGVHD grade 4 were skin, gastrointestinal tract, and liver. Of the 4 patients that developed aGVHD grade 4, one died and the remaining 3 recovered. However, one patient died of subsequent pneumonia 1.5 yrs after transplantation. One of the surviving 2 patients remained free of GVHD through the last follow-up of 634 days after transplantation, and the other one developed limited cGVHD at 484 days after transplantation and recovered 2 mos later.

Factors that might influence the incidence of GVHD grade 3-4 were analyzed in a univariate analysis that included age and gender of the patients and donors, number of MNC, CD3+, CD4+, CD8+, and CD34+ cells infused at transplantation, diagnosis stage of disease before transplantation, and HLA disparity. None of the factors was found to be significantly associated with the incidence of aGVHD grade 3-4.

Thirty-seven patients that survived longer than 100 days after transplantation were evaluated for cGVHD. Of 17 patients who had no cGVHD, 12 survived without relapse. Of 11 patients that developed limited cGVHD, 8 survived. The limited cGVHD involved skin in 6 patients and hepatic function in 2 patients; these were controlled with treatment for a median of 2 mos. Of 9 patients who developed...
extensive cGVHD, 7 survived. The extensive cGVHD included generalized skin and eye involvement in 3 patients, and involvement of skin, eye, and oral mucosa in 4 patients. The cumulative incidence of cGVHD was 56.7%, and that of extensive cGVHD was 29.5% (Figure 2). The development of cGVHD and its extensive type was not associated with the age or gender of the patients or donors, numbers of MNC, CD3⁺, CD4⁺, CD8⁺, or CD34⁺ cells infused at transplantation, the extent of HLA disparity, or the stage of disease before transplantation.

Nineteen opportunistic infections were observed within the follow-up time. The causes of opportunistic infection were pneumonia (6 cases), VZV (3 cases), bacteremia (Klebsiella Pneumoniae and Staphylococcus Aureus), infection in the gastrointestinal tract (CMV), and infection in the central nervous system (Aspergillosis). The causes of pneumonia were CMV in one case, fungi in one case (Aspergillosis), bacteria in one case (Staphylococcus Aureus), and idiopathic interstitial pneumonia in 3 cases. CMV antigenemia was detected in 18 patients; however, only one case of interstitial pneumonia (IP) was associated with CMV. Epstein-Barr virus (EBV) antigenemia was detected in 2 children and one of these died of EBV-associated lymphoproliferative disorder.

Hemorrhagic cystitis developed in 6 children at 25 to 160 (median 40) days after transplantation, and lasted for 2 to 6 (median 3.5) wks. Seizures occurred in 3 cases within 2 mos after transplantation with no abnormalities found in magnetic resonance imaging of brain or cerebral electric examinations. However, at the time of occurrence, serum CsA concentrations were at a minimum of 132ng/ml and 287ng/ml (normal range: 150-250 ng.ml), respectively. After the discontinuance of intravenous CsA, the seizures were resolved.

Table 3. Time of neutrophil and platelet recovery in patients with different extent of HLA disparity receiving haploidentical HSCT

<table>
<thead>
<tr>
<th>Mismatched loci (case)</th>
<th>Patients achieving neutrophil recovery at day 30 (case)</th>
<th>Patients achieving platelet recovery at day 30 (case)</th>
<th>Patients achieving platelet recovery at day 60 (case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-loci, n=23</td>
<td>23</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Three-loci, n=15</td>
<td>15</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Four-loci, n=4</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Five children without GVHD were followed up for T cell subset recovery in the blood. At 3 mos after transplantation the median CD4⁺ and CD8⁺ cell counts were 149.78 and 310.24 cells/µl, respectively. At 1 year, data were only obtained in 3 children and CD4⁺ and CD8⁺ cell counts were 382.00 and 1066.01 cells/µl, respectively. Of the 5 children without GVHD, 4 children survived free of relapse without any complications for 332, 788, 802, and 833 days after transplantation, and one child died of heart failure.

Table 4. Recovery of T cell subsets in the blood in children receiving haploidentical HSCT

<table>
<thead>
<tr>
<th>Median of T-cell counts/Months after transplantation</th>
<th>Before Conditioning (n=5)</th>
<th>1M (n=5)</th>
<th>3M (n=5)</th>
<th>6M (n=5)</th>
<th>12M (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3⁺ (cells/µl)</td>
<td>302.23</td>
<td>89.05</td>
<td>492.12</td>
<td>690.02</td>
<td>1264.66</td>
</tr>
<tr>
<td>CD4⁺ (cells/µl)</td>
<td>146.24</td>
<td>40.18</td>
<td>149.78</td>
<td>170.94</td>
<td>382.00</td>
</tr>
<tr>
<td>CD8⁺ (cells/µl)</td>
<td>127.72</td>
<td>58.36</td>
<td>310.24</td>
<td>588.61</td>
<td>1066.01</td>
</tr>
</tbody>
</table>

Figure 1. (A) Cumulative incidence of acute GVHD grade 2-4 with HLA disparity after haploidentical HCT for children. Among HLA-A, B, DRB1, 4 patients with 1 mismatched locus, 15 patients with 2 mismatched loci and 23 with 3 mismatched loci. Tick marks indicate censored patients. (B) Cumulative incidence of acute GVHD grade 3 and 4 with HLA disparity after haploidentical HCT for children. Tick marks indicate censored patients.
Survival and Relapse

Up to November 1, 2007, 27 patients survived. Twenty-five were free of leukemia: 10 patients with ALL survived through a median of 539 (40-1478) days, 10 with AML survived through a median of 663 (119-1629) days, and 5 with CML survived through a median of 780 (598-1337) days. Eighteen of the 25 patients were classified as high-risk candidates before transplantation with a 52.2\(\pm\)9.5% probability of LFS. Seven out of 9 patients in the standard-risk group survived free of leukemia. The 3-yr probability of LFS for all patients was 57.3\(\pm\)8% (Fig 3a). The quality of life of the survivors was evaluated with the Lansky Play-performance scale. Five patients that scored 70% were under treatment for extensive GVHD. Of 3 patients that scored 80%, 2 developed cGVHD. Twelve patients scored 90% and 7 patients scored 100%.

Fifteen patients died. Seven patients died from relapse of leukemia at a median of 476 (160-706) days after HSCT. Four patients died from infection: 2 from pneumonia on day 300 and 463, one from cytomegalovirus interstitial pneumonia on day 68, and one from cerebral fungal infection on day 170. Two patients died from heart failure on days 40 and 184; both had received chemotherapy before transplantation for 2 and 4 yrs, respectively. One died from severe aGVHD on day 67. Another died from EBV-associated lymphoproliferative disorder on day 55. The transplantation related mortality was 9.5\(\pm\)4.5% at 100 days and 20.4\(\pm\)6.5% at 1 yr after transplantation.

All 9 of the patients who relapsed after HSCT were diagnosed with ALL before transplantation, and 5 were from the high-risk group. The probability of relapse for high-risk patients was 37\(\pm\)10.2% at 2 yrs after transplantation (Figure 3b). 2 patients experienced hematological relapses of leukemia at 180 days and 120 days after transplantation; they received chemotherapy followed by donor lymphocyte infusions (DLI). They achieved complete remission and 100% donor chimerism 4 wks after DLI and were free of disease recurrence at follow-ups 1185 days and 1024 days after transplantation.

The univariate analysis identified several clinical factors that were associated with relapse and LFS after transplantation, including: age of the patients (P=.567 and .678), gender of the patients (P=.672 and .383), gender pair of the patients and donors (P=.685 and
numbers of cell subsets infused at transplantation (TNC: P = .536 ±.827; CD34+: P = .681 ±.932; CD3+: P = .278 ±.449), risk stratification of disease before transplantation (P = .445 ±.251), time between diagnosis and transplantation (P = .339 ±.600), time of myeloid recovery (P = .310 ±.779), time of platelet recovery (P = .812 ±.640), HLA disparity (P = .068 ±.427), occurrence of aGVHD grade 2-4 (P = .832 ±.629), occurrence of extensive cGVHD (P = .080 ±.631), and occurrence of opportunistic infections (P = .238 ±.860). In addition, a diagnosis of ALL was associated with a lower probability of LFS (Figure 4a, P = .008) and a higher probability of relapse (Figure 4b, P = .008) after transplantation. The occurrence of cGVHD was associated with a lower rate of relapse after transplantation (P = .019) (Figure 5). Patients with doses of CD34+ < 2 × 10^6 cells/kg and >2 × 10^6 cells/kg had 50.2 ±12.7% and 68.9 ±9.9% probabilities of LFS, respectively (P = .352), and no adverse influences were observed on relapses, GVHD, or opportunistic infections.

**DISCUSSION**

The prognosis for childhood hematological malignancies has improved dramatically over the past quarter of a century. A recent comparative study for allogeneic transplantation in children demonstrated that the 5-yr LFS rates were 38% after HLA-matched bone marrow transplants, 60% after HLA-matched CBT, and 33%-45% after mismatched CBT. However, CBT offered no advantage in platelet and neutrophil recovery, occurrence of cGVHD, TRM, or survival compared with the bone marrow transplantation from identical siblings [3]. Recently, we described a HLA-mismatched/haploidentical T cell replete HSCT (n = 171, 149 adults) for treatment of leukemia that achieved results comparable to transplantation from identical siblings [5,6]. However, to date, in most transplantation centers HSCT from mismatched family members is performed only in the context of a primary clinical trial and has not been recommended for patients with no identical sibling donors. The current study investigated the efficacy and toxicity of haploidentical HSCT as a leukemia treatment for children under 14 yrs old, most with high-risk hematological malignancies.
Our results showed incidence rates of 13.8% for acute grade 3-4 GVHD, 56.7% for cGVHD, and 29.5% for extensive cGVHD in children after haploidentical HSCT. These results were comparable to those reported in studies that used HLA-identical and unrelated donors for HSCT [1,10]. The results reported here in children were lower than those reported previously in adults receiving HLA-mismatched/haploidentical T cell replete HSCT (23.1%, 73.6% and 46.9%, respectively) [6]. Consequently, the quality of life after haploidentical HSCT for the children was much better than that of the adults.

Presently, more than 70% of children with newly diagnosed ALL that were treated with multiagent chemotherapy with or without radiotherapy survived and are disease free 5 yrs after treatment [11]. However, patients that are in CR1 with a high risk of failing current chemotherapy regimens or relapse within the first year of diagnosis require alternative treatment strategies [12]. Treatments with matched related allogeneic HSCT had previously been restricted to children who relapsed during or after primary intensive chemotherapy. The 5-yr LFS rate was 29% after matched related allogeneic HSCT for children that had ALL and an initial bone marrow relapse within 12 mos of completion of primary therapy [13]. Another study showed that children with ultra-high-risk features of ALL (Philadelphia chromosome, induction failure, age ≥10 yr, and WBC count ≥200,000/mm3) may benefit from transplantation in CR1, especially patients with primary induction failure and Philadelphia chromosome; the 5-yr LFS rate was 58.6% for these patients after HSCT from matched family donors [14]. In our present study, the 3-yr LFS rate was 52.2 ± 9.5% for children with high-risk leukemia, including patients with acute leukemia in CR1 or CR2 with Philadelphia chromosome, in remission after CR3, or in a non-remission state. Though in most transplantation centers unrelated donor or cord blood transplants were recommended for children with high-risk ALL who did not have identical sibling donors, the data in the present study encourage the use of haploidentical family member donors, even for children with ALL that are in CR1 or CR2, but who have poor risk features.

Relapse and heart failure were the main causes of mortality after transplantation. All 9 children in this study who relapsed after transplantation were diagnosed with ALL and were classified as high-risk candidates. Two children died of heart failure early after transplantation; this suggests that it is important to make timely choices to avoid unnecessarily long courses of conventional therapy that might result in irreversible organ injury. The incidence of opportunistic infections observed with our transplantation regimen was less than that reported with the Perugia regimen and T cell depleted grafts; this suggests that overall post-transplantation T cell reconstitution is significantly better with our regimen than that reported by Waller et al [15,16]. The limited data of the present study show that, in the 5 patients without GVHD or other complications, T cell and T cell subset recovery was at least comparable and possibly faster than the recovery rate reported previously, which indicated the potential role of the age of these patients in immune reconstitution [17-18].

Future research on children with high-risk leukemia might address the following questions: what is the role of stratifying patients into risk groups, including the role of cytogenetic screening, in selecting candidates for allogeneic HSCT, especially those in CR3? What is the optimal timing and appropriate use of haploidentical donor HSCT, given that a matched related donor is not available?

An evidence-based review published recently evaluated the role of HSCT as a therapy for children with AML. It showed that allogeneic HSCT from identical sibling donors resulted in superior overall survival and DFS rates compared to autologous HSCT and chemotherapy for children in CR1 or CR2 [19]. In a study by Alonzo et al., the 8-yr LFS for children with AML-CR1 was 47±5% after allogeneic HSCT with identical sibling donors (n=373), 42±7% after autologous HSCT (n=217), and 34±4% after chemotherapy alone (n=688) (P=.004) [20]. They recommended a matched unrelated or other alternative donor should be used for HSCT in the context of a clinical trial. In our study, 10 of 12 children with AML (5 children were in CR1) achieved LFS through 663(119-1629) days. Though the number of cases was limited, the primary data demonstrated the safety and efficacy of haploidentical HSCT for children with AML.

CML constitutes 3-5% of all childhood leukemias. Though the treatment of CML with tyrosine kinase inhibitor has been proven successful for several years, allogeneic HSCT is the only proven curative treatment for children with CML. In our study 5 out of 6 children with CML achieved LFS for a median of 217(D=217) days. Though the number of cases was limited, the primary data demonstrated the safety and efficacy of haploidentical HSCT for children with AML.

The use of family donors with 2 to 3 mismatched loci is considered a viable approach, despite the prompt availability of another suitable donor. This is based on the results observed in the high-risk group, where 9 children received haploidentical HSCT under the fervent request of their parents. Two children with CML had an indication for haploidentical HSCT due to the lack of identical sibling donors. Out of the 9 children, 3 died of infection or GVHD after transplantation. This indicated that children with standard-risk leukemia should not be
recommended for haploidentical HSCT. Though the results of this study should be interpreted cautiously due to the small number of patients, the primary results from children with high-risk leukemia suggested the feasibility of haploidentical HSCT in cases where no HLA identical donors are available. Although prospective, randomized studies are needed to address the more complex issues, the results described here support the use of haploidentical family members as donors. This strategy is likely to be most appropriate for patients who have no siblings.

REFERENCES