Outcome of Pediatric Hematopoietic Stem Cell Transplantations from Thai Unrelated Donors Matched with High-Resolution HLA Typing

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The authors evaluated the outcome of ten children given hematopoietic stem cell transplantations from Thai unrelated donors (URD-HSCT) selected using DNA high-resolution typing of both HLA class I and II loci. Six patient/donor pairs (60%) were fully matched; four (40%) were 5/6 matched. Patients had either non-malignant (n=9) or malignant (n=1) diseases. In most cases, graft-versus-host disease (GVHD) prophylaxis composed of cyclosporine and short-term methotrexate. The probability of hematopoietic recovery at day 30 was 90%. The cumulative probability of acute GVHD and of chronic GVHD equaled 44.4 and 0%, respectively. Three patients died of transplant-related complications. The probability of transplant-related mortality (TRM) at 30, 100, and 180 days were 10, 30, and 30%, respectively. The overall and disease-free survival rates were 70 and 70%, respectively. URD-HSCT with donor selection based on high-resolution HLA typing is associated with a low incidence of both severe acute GVHD and graft failure. The observed outcome is comparable to that of children transplanted from HLA-identical siblings.

Keywords: *Unrelated donor, Stem cell transplantation, HLA, Acute GVHD, Graft failure*

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Although hematopoietic stem cell transplantation from an unrelated donor (URD-HSCT) is an accepted mode of treatment of patients with hematological disorders, a higher risk of transplant-related mortality (TRM) has been reported when compared with transplantation from HLA-identical siblings.[1,2] The difference is, at least partially, because of HLA disparities between the recipient and the unrelated donor, sometimes not revealed because of the limits of serological or low-resolution molecular typing of HLA loci. These disparities contribute to an increased incidence of severe graft-versus-host disease (GVHD) and graft rejection, two major obstacles for successful allogeneic HSCT.[3-6] Therefore, adequate HLA matching seems to be essential to improve the outcome of URD-HSCT, and the results of such transplants must be assessed with consideration to the typing method used to select the most suitable donor.

Until recently, HLA matching was performed employing serological techniques for HLA-A and -B loci, and high-resolution molecular typing for HLA-DRB1 alleles. Many studies demonstrated that among serologically matched unrelated patient/donor pairs a high level of HLA class I incompatibility can be detected when DNA-based typing is applied.[7,8] Efforts have been made to reveal whether HLA class I allele disparity has an impact on clinical outcome. Thus, the authors analyzed the outcome prospectively. The recent study by the Seattle group demonstrated that multiple HLA class I and/or class II mismatches were associated with increased mortality, whereas patients transplanted with a single antigen-disparate donor had an outcome comparable to that of subjects given a fully matched transplant.[9] Two further studies including a smaller number of patients revealed that the presence of any mismatch for HLA class I or class II alleles was associated with a decreased probability of

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survival, compared with fully matched patient/donor pairs.[10,11] Taken together, all the above mentioned provided the rationale for introduction of high-resolution HLA class I molecular typing as a routine method in the process of unrelated donor selection.

In the present study, the authors assess the outcome of URD-HSCT in pediatric patients for whom the donor was prospectively selected using high-resolution DNA typing for both HLA class I (HLA-A, -B) and class II (HLA-DRB1) loci. The presented analysis mainly focused on the occurrence of both acute GVHD and graft failure (primary objectives). TRM, overall survival (OS) and event-free survival (EFS) were also analyzed, together with the potential influence of HLA incompatibility on the outcome.

Material and Method

Patients

Ten consecutive patients aged less than 15 years old treated with Thai URD-HSCT between May 2001 and June 2005 were included in the present study. The transplant procedures were performed in the HSCT center: Department of Pediatrics, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. All the donors were prospectively selected using DNA high-resolution typing for both HLA class I and class II loci. The source of stem cell consisted of 5 bone marrow (BM); 2 peripheral blood stem cells (PBSC); and 3 umbilical cord blood (UCB). The median age of the 9 male and 1 female patients was 6 years (range 1.25 year to 14 years), whereas the median age of 7 stem cell adult donors was 31.5 years (range 19 to 45 years). There were 3 cord blood units collected from 3 term neonates when they were delivered. Indications for URD-HSCT were β-thalassemia/hemoglobin E (n=5), adrenoleukodystrophy (ALD) (n=2), Wiskott-Aldrich syndrome (WAS) (n=1), severe aplastic anemia (SAA) (n=1) and acute lymphoblastic leukemia in third complete remission (ALL CR3) (n=1). Details on patient and donor characteristics are reported in Table 1. Patients and/or their parents signed informed consent before treatment. Thai volunteer donors or parents of cord blood donors also signed informed consent before donation.

HLA typing and matching

Unrelated donors were searched through the Thai national stem cell donor registry and the Thai national cord blood bank.[12,13] In all cases, HLA class I (HLA-A, -B) and class II (HLA-DRB1) loci were identified by high-resolution DNA typing using the commercial kit: PEL-FREEZ Clinical System LLC., Brown Deer, WI USA for HLA-A and –B, and ONE LAMBDA INC., Canoga Park, CA USA for HLA-DRB1. In 6 patient/donor pairs were considered fully allele-match. Among the other 4 patients for whom a fully matched unrelated donor could not be found, bone marrow transplantation (BMT) was performed despite the presence of one-allele mismatch in one case (SAA), and umbilical cord blood transplantation (CBT) was done despite one-allele disparities in three cases (1 WAS, 2 thalassemias). Disparities in the GVHD direction (recipient's antigen/allele not shared by the donor) were present in 3 out of 4 patients; disparities in the graft failure direction (donor's antigen/allele not shared by the recipient) were found in 4 out of 4 patients. All patients were transplanted from a donor matched at the DRB1 loci.

URD-HSCT procedure

Bone marrow was used as a source of hematopoietic stem cells in 5 patients, whereas granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood were transplanted in 2 cases. Umbilical cord blood was used in 3 cases.[12] In 7 patients given transplant of BM or PBSC, the median nucleated cell (NC) dose was 5.5 (range 2.6 to 10.9) x 10^8/kg, the median CD34+ cell dose was 5.4 (range 2.9 to 18) x 10^6/kg. In 3 patients given transplant of UCB stem cells, the median NC dose was 5.66 (range 2.15 to 44) x 10^7/kg, the median CD34+ cell dose was 3.0 (range 2.26 to 25) x 10^5/kg. The 5 thalassemia and 1 WAS patients were given busulfan (Bu) + cyclophosphamide (Cy) + anti-thymocyte globulin (ATG)[14] as preparative regimen, while the SAA patient received Cy + rabbit ATG, and the ALL (CR3) patient received non-myeloablative conditioning regimen [15-18] consisting of fludarabine + Bu + Fresenius ATG. Regarding the ALD patient number 1, the first conditioning consisted of Cy + equine ATG + melphalan. After unsatisfactory engraftment, he was re-transplanted from the same donor's cryopreserved PBSC using Cy + rabbit ATG + total body irradiation (TBI). The ALD patient number 2 received Bu + Cy + fludarabine + rabbit ATG as conditioning regimen.

Prophylaxis of GVHD consisted of cyclosporine and short-term methotrexate in BM and PBSC transplant patients. It consisted of cyclosporine +/- corticosteroids in UCB transplant patients. Methylprednisolone was used as the first-line therapy of acute GVHD.

All patients were given recombinant human G-CSF after transplantation. It was administered starting from 4 hours after URD-HSCT and continued once

daily until neutrophil recovery (> 2 x 10^9/l). Cytomegalovirus (CMV) serological status was studied before transplantation in all donor/recipient pairs (Table 1) and CMV DNA viral replication was monitored in all patients post-transplant in order to detect CMV reactivation. Patients experiencing reactivation of CMV infection were treated according to a strategy of pre-emptive therapy with ganciclovir at conventional dosage until antigenemia became negative.[19]

Definition

Acute GVHD was diagnosed and graded according to previously reported criteria.[20] All patients surviving more than 7 days after transplant were considered at risk for developing acute GVHD. Children living 100 days post-transplant with sustained donor engraftment were considered to be evaluable for chronic GVHD, which was classified as previously described.[21]

Neutrophil engraftment was defined as the first day of an absolute neutrophil count (ANC) > 0.5 x 10^9/l for 3 consecutive days. Platelet engraftment was defined as the first day of a platelet count > 20 x 10^9/l for 3 consecutive days without platelet transfusion support.

Graft failure or rejection occurred if ANC did not rise within 28 days after URD-HSCT or declined below 0.2 x 10^9/l after initial recovery. The rate of graft failure was defined as (100 - ANC engraftment rate (%)) at day 100 after transplantation.

TRM was defined as all causes of death without evidence of initial disease. OS was the time between transplantation and death due to any cause, whereas EFS was defined as time interval from HSCT to the first event (either disease recurrence or death without recurrence whichever occurred first).

Results

Engraftment

The ALD patient number 1 experienced mixed chimerism of recipient predominance after first PBSC transplantation, then needed to undergo second transplantation from the same donor's cryopreserved stem cells one year later. The result of the second transplant was full donor engraftment. The SAA patient died at day 4 post-transplant before achieving engraftment. The remaining 8 patients were all engrafted. Median time to reach neutrophil and platelet recovery was 15 and 28 days, (range 11 to 19, and range 13 to 41) respectively. The probability of hematopoietic recovery at day 30 equaled 90% (9 out of 10 patients). Successful donor engraftments were proven by chimerism analysis using microsatellite method.

Table 1. Patient and donor characteristics

	Cases (total n = 10)
Median patient age (range)	6 (1.25-14) years
Median donor age (range) (n = 7)	31.5 (19-45) years
Patient/donor sex	
Male/male	7
Male/female	2
Female/female	1
Patient/donor CMV status	
Positive/positive	8
Negative/positive	1
Negative/negative	1
Diagnosis and disease status at URD-HSCT	
β-Thalassemia/hemoglobin E	5
Adrenoleukodystrophy	2
Severe aplastic anemia	1
Acute lymphoblastic leukemia (CR3)	1
Wiskott-Aldrich syndrome	1

Graft-versus-host disease

Three patients developed grades II-IV acute GVHD and one patient had grade III-IV GVHD. The cumulative probability of developing this complication at day 100 equaled 44.4% (4 out of 9 patients). However no patients died of acute GVHD. All affected patients responded well to either first- or second-line therapy. Among the four patients who developed acute GVHD, one child (thalassemia) was transplanted from HLA-matched BM; one (thalassemia) from one-allele HLA-A mismatched UCB; one (thalassemia) from one-antigen HLA-B mismatched UCB; and one (ALD number 1) from HLA-matched second PBSC.

There was no chronic GVHD in the present series. All acute GVHD cases were treated and resolved completely prior to day 100. Details on acute GVHD according to HLA matching are reported in Table 2.

Transplant-related mortality

Three patients died of transplanted-related complications, one child (SAA) died at day 4 due to severe neutropenic septicemia; the other two (ALL and thalassemia) despite achieving full donor engraftment each died in their third month post-transplant due to severe septicemia and cardio-respiratory failure. The overall probability of TRM equaled 30%.

Table 2. Outcome of URD-HSCT with respect of donor/recipient HLA matching

	Fully matched (n=6) (Cases) (%)		Single mismatch (n=4) (Cases) (%)	
Acute GVHD grades II-IV	2	(33.3)	1	(25)
Acute GVHD grades III-IV	0	(0)	1	(25)
Chronic GVHD	0	(0)	0	(0)
Overall survival	5	(83.3)	2	(50)
Event-free survival	5	(83.3)	2	(50)
Transplant-related mortality	1	(16.7)	2	(50)

Survival

The OS and EFS of all patients were the same 70% (7 out of 10). Median follow-up time for surviving patients was 1 year 3 months (range 9 months to 3 years 2 months).

Discussion

To date the present study is the first and largest case-series report of HSCT from Thai unrelated donors. The present study provides the evaluation of patients for whom donor search and selection was based prospectively on high-resolution typing for both HLA class I and class II loci. The incidence of grade II (mild to moderate form) and grade III-IV (moderate to severe form) acute GVHD in the presented group were comparable to other studies. No patients died of GVHD. This finding together with the low incidence of graft failure (10%) translated into a low probability of early (before day 100) (30%) and overall (30%) TRM. The main cause of mortality was more frequently associated with the toxicity of myeloablative therapy than with immunological complications.

In the study by the National Marrow Donor Program analyzing 363 children with ALL in second complete remission, the probability of grade III-IV acute GVHD equaled 29% with a TRM rate of 42%.[22] Smith et al[23] reported the outcome of 46 patients with juvenile myelomonocytic leukemia who underwent URD-HSCT and the results of grades II-IV acute GVHD was 73%. The European Group for Blood and Marrow Transplantation reported that 34% of 69 Fanconi's anemia patients who underwent transplantation developed grade III-IV acute GVHD.[24] In all the above studies, serological typing of HLA class I antigens was used for donor selection. The use of DNA high-resolution typing for both HLA class I and II alleles appears to be an important factor contributing to reduction of immunological complications in children undergoing URD-HSCT. With more accurate HLA matching, the probability of finding a fully compatible unrelated donor is decreased.[25] This implies that some level of incompatibility must be accepted when compared to a proportion of patients who are in need of URD-HSCT. In the present study, the authors did not find any significant correlation between the presence of HLA incompatibility in the donor/recipient pairs and the treatment outcome. However, this could be related to the limited number of subjects and events.

In a cohort of 300 patients, Petersdorf et al[9] demonstrated that a single HLA mismatch did not influence the incidence of both severe acute GVHD and mortality. On the other hand, HLA-C disparity was found to increase the risk of graft failure.[4] Graft failure appeared to be strongly dependent on the stem cell dose, as well as on the recipient age, both factors being in favor of pediatric patients.[26] This advantage may overcome the HLA disparity-related risk of graft failure.

All the patients treated in the Seattle study were not given pre-transplant ATG. In contrast, in the presented study group, all patients received conditioning regimen composing ATG that may also have the effect of GVHD prophylaxis. This difference seems to be important, since ATG has recently been proven to reduce the incidence of both acute and chronic GVHD in a dose-dependent manner.[27] This kind of serotherapy leading to a depletion and modulation of function of donor T cells may be essential for overcoming the risk of immunological complications related to HLA incompatibility between donor and recipient.

Conclusion

Donor selection based on high-resolution DNA typing for both HLA class I and class II loci, seems to improve the outcome of patients undergoing URD-HSCT. Using this approach for donor selection, the risk of immunological complications, as well as TRM, is similar to that observed after transplant from a genotypically identical sibling. If a perfectly matched donor is not available, the presence of a single

mismatch, or even double disparity in cord blood, is acceptable and does not seem to influence the outcome in pediatric patients.

Further attempts should be focused on distinguishing permissible from non-permissible mismatches, as well as on the evaluation of the role played by minor histocompatibility antigens reported to influence the risk of GVHD in some retrospective analyses.[28] Finally, further large studies may provide information concerning the significance of particular mismatches.

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ผลการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตในผู้ป่วยเด็กจากผู้บริจาคคนไทยที่คัดเลือกโดยวิธีตรวจ HLA แบบ high-resolution

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ผู้รายงานประเมินผลการรักษาผู้ป่วยเด็ก 10 รายด้วยการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตจากผู้บริจาคคนไทยที่ไม่ใช่ญาติพี่น้อง โดยคัดเลือกผู้บริจาคด้วยการตรวจ HLA ทั้ง class I และ II แบบ high-resolution DNA typing พบว่ามีผู้ป่วยและผู้บริจาค 6 คู่ (ร้อยละ 60) มี HLA ตรงกันหมด ผู้ป่วยและผู้บริจาค 4 คู่ (ร้อยละ 40) มี HLA เข้ากันได้เพียง 5 ใน 6 ตำแหน่ง ผู้ป่วยเด็กเป็นโรคที่ไม่ใช่มะเร็ง 9 ราย เป็นโรคมะเร็ง 1 ราย ผู้ป่วยส่วนใหญ่ได้รับยาป้องกันการเกิดภาวะ graft-versus-host disease (GVHD) ด้วย cyclosporine และ methotrexate ระยะสั้น ผู้ป่วยร้อยละ 90 มีการปลูกถ่ายเซลล์ติดเมื่อนับถึงวันที่ 30 ผู้ป่วยร้อยละ 40 เกิดภาวะ GVHD แบบเฉียบพลัน แต่ไม่มีผู้ป่วยเกิดภาวะ GVHD แบบเรื้อรัง ผู้ป่วย 3 ราย (ร้อยละ 30) เสียชีวิตจากผลข้างเคียงของการปลูกถ่าย อัตราการรอดชีวิตและการรอดชีวิตโดยปราศจากโรค ต่างเท่ากับร้อยละ 70 การปลูกถ่ายเซลล์ต้นกำเนิดเม็ดโลหิตจากผู้บริจาคคนไทย โดยคัดเลือกผู้บริจาคด้วยการตรวจ HLA แบบ high-resolution DNA typing สัมพันธ์กับอุบัติการณ์ต่ำของทั้งการเกิด GVHD แบบรุนแรงและการปลูกถ่ายเซลล์ไม่ติด ผลการรักษาโดยการปลูกถ่ายเซลล์จากผู้บริจาคคนไทยนี้ ได้ผลดีทัดเทียมกับการปลูกถ่ายจากพี่น้องที่มี HLA ตรงกัน