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Brief Articles

Sufficient Immunosuppression with Thymoglobulin Is Essential for a Successful Haplo-Myeloid Bridge in Haploidentical-Cord Blood Transplantation

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A B S T R A C T

In haploidentical (haplo)-cord blood (CB) transplantations, early haplo donor engraftment serves as a myeloid bridge to sustainable CB engraftment and is associated with early neutrophil recovery. The conditioning regimens as published for haplo-cord protocols usually contain serotherapy, such as rabbit antithymocyte globulin (ATG) (Thymoglobulin, Genzyme, Cambridge, MA). However, reducing or omitting serotherapy is an important strategy to improve early immune reconstitution after transplantation. The need for serotherapy in successful haplo-cord transplantation, defined as having a haplo-derived myeloid bridge to CB engraftment, has not been investigated before. Two consecutive cohorts of patients underwent transplantation with haplo-CB. The first group underwent transplantation with haplo-CB for active infection and/or an underlying condition with expected difficult engraftment without a conventional donor available. They received a single unit (s) CB and haplo donor cells ($CD34^+$ selected, 5×10^6 $CD34^+$/kg). The second cohort included patients with poor-risk malignancies, not eligible for other treatment protocols. They received a sCB and haplo donor cells (CD19/αβTCR-depleted; 5×10^6 $CD34^+$/kg). Retrospectively in both cohorts, active ATG (Thymoglobulin) levels were measured and post–hematopoietic cell transplantation area under the curve (AUC) was calculated. The influence of ATG exposure for having a successful haplo-myeloid bridge (early haplo donor engraftment before CB engraftment and no secondary neutropenia) and transplantation-related mortality (TRM) were analyzed as primary endpoints. Twenty patients were included (16 in the first cohort and 4 in the second cohort). In 58% of evaluable patients, there was no successful haplo-derived myeloid bridge to CB engraftment, for which a low post-transplantation ATG exposure appeared to be a predictor ($P < .001$). TRM in the unsuccessful haplo-bridge group was 70% ± 16% versus 12% ± 12% in the successful haplo-bridge group ($P = .012$). In conclusion, sufficient in vivo T depletion with ATG is required for a successful haplo-myeloid bridge to CB engraftment.

INTRODUCTION

Hematopoietic cell transplantation (HCT) is a curative treatment option for a variety of malignant and nonmalignant diseases in adults and children. Although HCT has become safer over the last decade, the major limitations remain relapse and transplantation-related mortality (TRM), due to infection, viral reactivations/disease and graft-versus-host disease (GVHD). Unrelated cord blood (CB) is an emerging alternative stem cell source for HCT, as it has many advantages compared with conventional sources, such as marrow and peripheral blood. These include prompt availability and less stringent HLA-matching criteria resulting in increased donor availability [1] and a lower probability of GVHD, notwithstanding a

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powerful graft-versus-leukemia effect [2-4]. On the other hand, the cell number in the unit may be a limiting factor associated with a prolonged neutropenia after CB transplantation [5-7]. Prolonged neutropenia and lymphopenia are important predictors for increased TRM [8-10]. Combining a $CD34^+$-selected haploidentical (haplo)-graft with a full graft CB unit is a recently introduced cell support procedure to make single CB (sCB) as donor source available to a larger proportion of patients [11-13]. It has been shown that early haplo-donor cell engraftment can shorten neutropenia and, as such, serve as a myeloid bridge until followed by sustainable CB engraftment. In several reports, this resulted in a fast (<14 days) neutrophil engraftment [11-13]. In a Spanish study, 86% of patients ultimately reached full CB donor chimerism within the time frame of 11 to 186 days after transplantation (median, 44 days), suggesting that the haplo stem cell graft can only serve as temporary bridge to full engraftment of an optimally selected CB unit. Interestingly, the acute GVHD rates were low [11,12]. Similar results were reported from a cohort in Chicago [13] and a for a cohort of patients with aplastic anemia [14]. In all of these described cohorts, antithymocyte globulin (ATG) was part of the conditioning regimen. Because of its long half-life, ATG will most certainly hamper the early T, B, and NK cell reconstitution of the engrafted donors and was, for that reason, reduced in dose in many different (including CB) transplantation settings [15]. On the other hand, ATG may support engraftment of T cell–depleted (haplo) products. Many specifically haplo transplantation centers have moved to other strategies of in vivo T cell depletion, such as post-HCT cyclophosphamide, in an attempt to still secure engraftment but improve immune reconstitution, as well [16-18].

The early neutrophil recovery in haplo-cord transplantation is caused by a secure haplo-derived myeloid bridge until sustained CB engraftment occurs. Having used more variable (and earlier) ATG timing in our center, we were able to investigate the impact of ATG exposure to the graft on the success of this haplo-myeloid bridge based on serial sampling and pharmacokinetic modeling [19].

METHODS

Setting and Study Population

Since 2009 in the University Medical Center Utrecht, 2 haplo CB protocols were open.

Cohort A (single CB/$CD34^+$–selected haplo)

Patients with any HCT indication with active infection and/or known difficult engraftment (severe aplastic anemia, osteopetrosis, hemophagocytic lympho-histiocytosis) and lacking a 10/10-matched sibling or 10/10-matched unrelated donor (instantly) available, were candidates to be enrolled in our sCB/$CD34^+$–selected haplo protocol (open since June 2009). Also, patients with only a sCB unit available with a lower than acceptable nucleated cell (NC) count (minimum total nucleated cell dose: 2.5, 3, and 5×10^7 NC/kg in case of a 6/6, 5/6, or 4/6 HLA-matched unrelated CB unit, respectively) were offered a sCB coinfused with a haploidentical graft (5×10^6 $CD34^+$/kg) [20].

Cohort B (sCB/CD19 and αβTCR-depleted haplo; NL31978.000.10)

Adults with poor-risk malignancies, not eligible for other treatment protocols, received a sCB + CD19/αβTCR-depleted haploidentical donor cells (also 5×10^6 $CD34^+$/kg). It was hypothesized that by using this depletion method, the haplo-innate cells (NK and gamma-delta T cells) are coinfused, which may have an additional antileukemic effect in the early phase after transplantation [20,21]. No ATG was given in this protocol to preserve the innate cells after transplantation.

All data were collected prospectively. Patients were enrolled in HCT and research protocols only after written informed consent and institutional ethical committee approval.

Donor Selection and Stem Cell Processing

CB units were selected based on HLA typing (minimum 4/6 match on intermediate resolution, HLA-A and -B on low resolution, and HLA-DR on high resolution) and cell count, with a lower acceptable limit of 1.5×10^7 NC/kg before cryopreservation. Haplo donors were selected based on their suitability to donate and undertake a peripheral blood stem cell collection procedure with granulocyte colony–stimulating factor mobilization. The related donors underwent stem cell mobilization with G-CSF (Filgrastim, 5 mcg/kg/day twice a day; Amgen, Thousand Oaks, CA, USA) for 4 consecutive days. Apheresis was started on day 5 and continued until at least 8×10^6 $CD34^+$ cells/kg recipient were harvested to take into account a loss with the selection and depletion columns.

For cohort A, after peripheral blood collection from the haplo donor, 5×10^6 $CD34^+$ cells/kg were selected and infused. $CD34^+$ cells were positively selected using the Miltenyi CliniMACs $CD34^+$ selection column (Miltenyi Biotec GmbH , Bergisch Gladbach, Germany). For cohort B, Miltenyi CD19/αβTCR beads were used to deplete B cells and αβ-T cells from the haplo donor graft, collecting also 5×10^6 $CD34^+$ cells/kg recipient. For cohort B, the maximum allowed number of αβT cells in the graft was $< 5 \times 10^4$/kg. The haploidentical graft was freshly infused within 1 to 5 hours after the infusion of the CB unit in both protocols.

Transplantation Details, Conditioning Regimen, and Supportive Care

The main myeloablative regimen contained Busulfan (Busilvex; Pierre Fabre, Boulogne-Billancourt, France) with therapeutic drug monitoring (target area under the curve [AUC] 90 to 95 mg*hour/L and fludarabine [Flu] 160 mg/m^2). A reduced-intensity alternative (adults) was Flu [cumulative dose 160 mg/m^2]/cyclophosphamide [60 mg/kg]/total body irradiation 2×200 cGy. For second HCTs (cohort A): Treosulfan (42 g/m^2 in 3 days [Medac, Wedel, Germany], Flu 160 mg/m^2).

Serotherapy (in cohort A) included Thymoglobulin (Genzyme Cambridge, MA; 10 mg/kg in 4 consecutive days: -9,-8,-7,-6). Alemtuzumab (Campath-1H; Genzyme) was given for second transplantations and patients previously receiving Thymoglobulin as part of the therapy for severe aplastic anemia.

Active Thymoglobulin (ATG) and alemtuzumab levels were measured retrospectively as described below and post-HCT serotherapy exposure (AUC) was determined [22]. Patients receiving alemtuzumab were not included in the analysis but are briefly described.

Patients received GVHD prophylaxis with cyclosporine A (trough level 200 to 250 mg/L) from day −2 to 3 months for malignant indications (6 months for nonmalignant). Prednisone 1 mg/kg was administered from days 0 to 30 as engraftment syndrome prophylaxis in pediatric patients. Patients received G-CSF (Filgrastim) 10 mcg/kg/day once daily i.v. from day +7 until neutrophil engraftment $> 2 \times 10^9$/L.

As antibacterial prophylaxis, patients received ciprofloxacin and cefazolin until neutrophil engraftment (>500/uL) and recovery of mucositis. Patients received viral prophylaxis with acyclovir and *Pneumocystis Jiroveci* prophylaxis with cotrimoxazole until 6 months after HCT. Seven of 16 patients in cohort A were on antifungal therapy because of active ongoing fungal infection. Therapy was preferably based on resistance patterns from fungal isolates. If not treated therapeutically, patients were considered to be at high risk for fungal infection and received prophylaxis with voriconazole as the first choice (trough level >1 mg/dL) [23,24]. Concomitant treatments of the adult patients have been described in more detail elsewhere [25,26].

Post-transplantation Follow-Up

Monitoring for Epstein-Barr virus, cytomegalovirus, and adenovirus DNA positivity was performed weekly as described elsewhere [27]. Chimerism studies were performed on unfractionated peripheral blood samples (L > .4) and repeated every 2 to 4 weeks until > 95% CB chimerism was observed.

The haplo-myeloid bridge to CB engraftment was considered successful if haplo-derived neutrophil engraftment would precede CB engraftment without an interval of cytopenia. Graft failure (GF) was defined as either (1) early loss of haplo and no engraftment of the CB, or (2) secondary loss of both grafts. Death without disease progression or relapse was considered transplantation related. Toxicity was scored according to National Cancer institute Toxicity Criteria; acute GVHD (aGVHD) was scored according to the Glucksberg criteria with the caveat of late aGVHD occurring after day 100, as described by the National Institutes of Health consensus guidelines [28,29]. Chronic GVHD was defined as stated in National Institutes of Health guidelines.

Analytical Assay

Active ATG, capable of binding to human T lymphoma cell line (HUT) cells, was measured using a quantitative flow cytometry assay [22,30]. In short, HUT-78 T cells were incubated with patient serum and subsequently with labeled fluorescent goat antirabbit IgG. For standard reference, HUT cells were incubated with known amounts of ATG (Thymoglobulin, Genzyme). Active ATG is measured in arbitrary units (AU), where the lower limit of quantification was .1 AU/mL.

Endpoints

Post-transplantation ATG-exposure (AUC: AU*day/L) has been shown to be of strong influence on immune reconstitution for HCT in pediatric patients and is investigated as a predictive factor for successful haplo-cord transplantation [19]. Primary endpoints included (1) successful haplo-myeloid bridge to *sustained CB engraftment*, which was defined as neutrophil engraftment without secondary neutropenia occurring, and at the time of engraftment a combined (haplo > CB) chimerism that over time switches to full (>95%) CB donor chimerism, and (2) TRM.

Other endpoints included overall survival (OS), GF, aGVHD, neutrophil and platelet engraftment (95% confidence intervals of neutrophil engraftment > 500/uL within 60 days, thrombocyte engraftment to 20×10^9/L within 180 days), and > 95% cord blood donor chimerism.

Statistical Analyses

For statistical analysis, follow-up time is the time to the last assessment for surviving patients or to the time of death. Logistic regression was used to visualize and analyze the binary data and to depict the chance of successful myeloid bridge as a function of post-HCT active ATG AUC. Kaplan-Meier estimates are calculated for nonrelapse mortality. We performed regression analysis on the primary endpoints for the variables of post-HCT ATG exposure, CB NC dose, stem cell transplantation number, HLA mismatch, age, and underlying condition. The association of a successful haplo-derived myeloid bridge for the TRM and GF were analyzed. Statistical analysis was performed using SPSS, (IBM, Inc.) software packages version 19.0 and R version 3.0.1. For pharmacokinetic analyses NONMEM 7.2 (Icon, Ellicott City, MD) was used.

RESULTS

Twenty-two patients were treated with haplo-CB transplantations (Table 1). The median age was 16.5 years (range, .25 to 59.1). Eighteen patients (15 children, 3 adults; 14 with active infection, 1 previous GF, 3 low NC-dosed CB) were included in cohort A: 11 patients had a nonmalignant indication for transplantation (7 immunodeficiencies, 1 osteopetrosis, 3 severe aplastic anemia) and 7 had a malignant indications (1 non-Hodgkin lymphoma, 1 acute myeloid leukemia, 3 acute lymphoid leukemia, 2 myelodysplastic syndrome). Three patients received haplo-CB as a second transplantation. Four patients with poor-risk malignant disease (Table 1) underwent transplantation in cohort B. For 3 of them, it was their second transplantation.

The median CB cell dose at selection was 4.13×10^7 NC/kg (range, 2.0 to 20.0) and 2.0×10^5 CD34$^+$cells/kg (range, .2 to 6.2). Six patients received a 6/6-matched CB unit, 12 patients received a 5/6 CB unit, and 4 patients a 4/6 CB unit.

In cohort A, 2 patients received alemtuzumab (Campath-1H) instead of ATG in their regimen (.5 mg/kg and .75 mg/kg) and 1 received no serotherapy. In cohort B, per protocol, none of the patients received serotherapy. For analysis of the Thymoglobulin impact, we only included patients in the 2 protocols who received either Thymoglobulin as serotherapy or no serotherapy at all (n = 20).

The minimum follow-up of the patients who remained alive is 18 months.

Primary Endpoints

One patient (patient number 5) was not evaluable for the primary endpoint of having a successful myeloid bridge because of early death after HCT (day +14). In 58% (11 of 19) of evaluable patients, there was no successful haplo-derived myeloid bridge to CB engraftment. None of the patients treated without ATG in the conditioning regimen (n = 5) had a successful haplo-myeloid engraftment. The median post-HCT active ATG AUC of patients receiving ATG was 17 AU*day/L (range, 0 to 139). Evaluating patients receiving either ATG (n = 14) or no serotherapy (n = 5, ATG AUC = 0) altogether, only ATG exposure appeared to be a predictor ($P < .001$) for successful haplo-myeloid engraftment

Table 1
Patient Characteristics

Patient No.	Age	Diagnosis	SCT No.	Indication for Haplocord	Cohort	Serotherapy	Haplo Selection Method
1	17 yr	NHL-CR3	First	Ongoing infection (Aspergillus)	A	ATG	CD34$^+$ selection
2	1 yr	HLH	First	Ongoing infection (Fusarium, pseudomonas)	A	ATG	CD34$^+$ selection
3	16 yr	AML-CR2	First	Ongoing infection (Aspergillus)	A	ATG	CD34$^+$ selection
4	17 yr	CID, autoimmune enteropathy	First	SIB cord low cell number, multiple viral infections, very high risk of GVHD	A	ATG	None (10/10 match)
5	3 mo	Osteopetrosis	Second	Difficult engraftment, progression of disease and treatment related morbidity in first SCT	A	ATG	CD34$^+$ selection
6	9 yr	ALL-CR2	First	Ongoing infection (Aspergillus)	A	ATG	CD19/CD3 depleted
7	14 yr	CID: Nemo deficiency	First	Ongoing infection (Aspergillus), CMV, EBV, VZV	A	ATG	CD34$^+$ selection
8	41 yr	Aplastic anemia	First	No conventional donor; aplastic for years	A	ATG	CD34$^+$ selection
9	18 yr	X-linked CGD	First	Ongoing colitis, unknown infections, low dose CB unit	A	ATG	CD34$^+$ selection
10	4 yr	ALL-CR1	First	Ongoing infection (Aspergillus)	A	ATG	CD34$^+$selection
11	6 yr	CGD	First	Ongoing infection (Aspergillus)	A	ATG	CD34$^+$ selection
12	15 yr	CID	First	Ongoing infection (Aspergillus)	A	ATG	CD34$^+$ selection
13	15 yr	Aplastic anemia	First	No conventional donor	A	ATG	CD34$^+$ selection
14	17 yr	ALL-CR3	First	Urgency, toxicity issues ALL therapy, ongoing infection (Aspergillus)	A	ATG	CD34$^+$ selection
15	12 yr	MDS-Raeb-T	Second	Ongoing infection (Aspergillus)	A	no	CD34$^+$ selection
16	52 yr	MDS + HIV	First	Ongoing infection, no donor	A	ATG	CD34$^+$ selection
17	49 yr	Third relapse ALL	Second	No available MUD donor	B	no	CD19/αβTCR depletion
18	46 yr	Second allo-sct CMML	Second	Second relapse	B	no	CD19/αβTCR depletion
19	38 yr	Second relapse Hodgkin	First	Second relapse	B	no	CD19/αβTCR depletion
20	59 yr	Second allo relapse AML	Second	Second relapse, no conventional donor	B	no	CD19/αβTCR depletion
21	5 yr	Kostmann, BM-failure	Second	Ongoing infection	A	Campath-1H	CD34$^+$ selection
22	17 yr	Aplastic anemia	First	No conventional donor, urgency	A	Campath-1H	CD34$^+$ selection

NHL indicates non-Hodgkin lymphoma; CR, complete remission; HLH, hereditary lymphohistiocytosis; AML, acute myeloid leukemia; CID, combined immunodeficiency; SIB, sibling; ALL, acute lymphatic leukemia; Nemo, NF-κB-essential modulator; CMV, cytomegalovirus; EBV, Epstein-Barr virus, VZV, varicella zoster virus; CGD, chronic granulomatous disease; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; HIV, human immunodeficiency virus; MUD, matched unrelated donor; allo, allogeneic, BM, bone marrow.
All patients who underwent transplantation with haplo-CB are shown. Patients who had alemtuzumab in the regimen who were excluded from the analysis are shown in grey.

(Figure 1A,B). TRM in the group not having a successful haplo-donor-derived myeloid bridge (n = 11) was 70% ± 16% versus 12% ± 12% in the group with a successful myeloid bridge (n = 8, $P = .012$) (Figure 1C). Analyzing only the patients in cohort A, this strong association between post-HCT ATG AUC and successful haplo-myeloid engraftment remained ($P < .001$).

For the 2 patients in cohort A who were excluded because they received alemtuzumab as their serotherapy agent (patient numbers 21 and 22) (Table 1), we observed the same association. One patient had a very high alemtuzumab exposure and had a successful haplo-derived myeloid bridge, and the other patient had a very low exposure and did not have a successful myeloid bridge. He, fortunately, had an early CB engraftment. Both patients are alive and well.

Secondary Endpoints

Overall, in our haplo-cord cohort there was a cumulative incidence (CI) of neutrophil recovery of 95% with a median of 13 days (range, 9 to 52). The CI of platelet recovery to 50 × 10^9/L at day 180 was 78% for evaluable patients. The median time to reach platelet recovery to 50 × 10^9/L was 26 (range, 11 to 88) days. In 87% patients without a successful myeloid bridge, there was a primary or secondary GF involving the ultimate loss of both grafts, explaining the high TRM. In the group with a successful myeloid bridge, we only observed a graft loss in 1 of 8 patients. OS in the patients receiving either Thymoglobulin or no serotherapy at all was 42% ± 12% after a median follow-up of 254 days (range, 14 to 2393). Corresponding with TRM, OS was much lower in patients without a successful myeloid bridge (OS of 25% ± 15% versus 82% ± 12% in patients with a successful haplo-derived myeloid bridge ($P = .021$) (Figure 1D).

In an attempt to exclude other causes of donor failure, the recipients with a loss of the haplo- and/or CB graft were tested for the development of HLA antibodies, which were not detected in any of them.

We evaluated the haplo to CB chimerism switch that is known to occur over time in haplo CB transplantations. Early, at 1 month after HCT, patients who undergo transplantation in the context of ATG (n = 14 with > 1-month survival) showed signs of significant haplo engraftment, with an initial chimerism of > 80% haplo donor in 11 of 14 patients. Eighty-three percent (10 of 12) of evaluable patients with more than 3 months of follow-up after haplo CB transplantation reached full donor CB chimerism (>95%) at a median of 90 days (range, 23 to 925) after HCT. In contrast, in patients without ATG exposure (n = 5), there was an early loss of—or

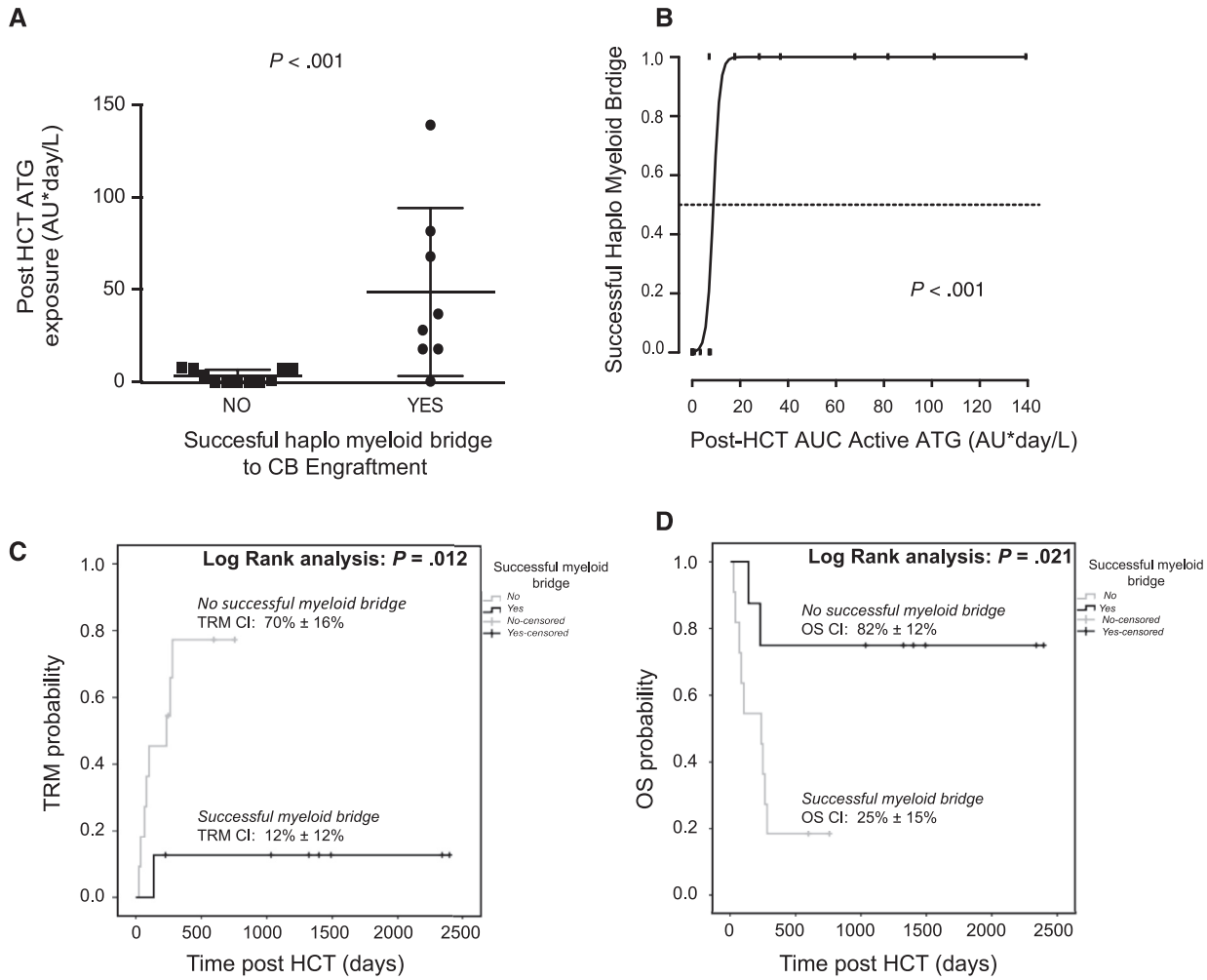

Figure 1. ATG exposure and outcome of haplo-cord transplantation. (A) Post-HCT AUC of active ATG (Thymoglobulin) of patients who underwent a haplo-cord blood transplantation, depicted for patients who did or did not have a successful haplo-derived myeloid bridge to cord blood engraftment. (B) A logistic regression depicting the chance of successful haplo-derived myeloid bridge as a function of the post-HCT AUC of active ATG. (C and D) Kaplan-Meier curves representing a comparison and log-rank analysis for the probability of (C) TRM and (D) OS for patients with and without a successful haplo donor-derived myeloid bridge.

a never even measurable—haplo-chimerism signal on whole blood. Instead, patients without ATG exposure showed full donor CB chimerism at a median of 24 days (range, 21 to 76) after HCT; however, most with low neutrophil counts.

Toxicity Endpoints

The CI of GVHD grade II to IV was 40% ± 12% (5 patients grade II, 2 patients grade III). However, specifically in patients treated without ATG, aGVHD grade II to IV occurred in 4 of 5 patients (grade II [n = 2], grade III [n = 1], grade IV [n = 1]). All had 100% CB donor chimerism at the time of GVHD diagnosis. However, because of the small sample size and "no ATG" occurring more in older patients with refractory malignancy treated in cohort B, definite conclusions cannot be made on this association.

No cases of veno-occlusive disease or other severe toxicity were observed. Four of the 7 patients with ongoing proven fungal infection were cured and antifungals were stopped successfully. In 2 other patients, fungal infections continued to be controlled with antifungals, which were continued until CD4 cell counts are recovered $> 200 \times 10^6$/L. In 10 of 19 patients, viral reactivation of cytomegalovirus (n = 9) or Epstein Barr virus (n = 1) occurred, which were successfully controlled with antivirals. Of the patients in cohort B with poor graft function and GVHD, 1 developed an Aspergillus infection.

DISCUSSION

Here, we describe the patients treated with haplo-CB transplantation in our center, in which different regimens for ATG dosing had been used. We observed a larger percentage of GF than previously published and investigated the role of ATG exposure to the security of the sequential engraftment of haplo and CB donors in the haplo-CB setting.

We find that the risk of nonengraftment of the haplo donor is substantially increased below a defined threshold of ATG exposure. Early rejection of the haplo donor was also associated with a primary or secondary failure of sustainable CB engraftment, potentially due to complex graft-versus-graft effects or associated with the high-risk nature of the patient selection, not allowing the wait for a long CB engraftment period. Consequently, we observed a higher TRM and lower OS, most likely due to prolonged neutropenia. We hypothesize, based on the chimerism data, which show a dominant CB signal in these cases, that rejection of the haplo donor is mediated by T cells from the CB unit.

Engraftment problems have not previously been raised as a major complication in the earlier described haplo-CB cohorts and the reported GF rates are between 7% and 15%. However, all published studies describing haplo-CB transplantation were performed in adults in the context of ATG administered shortly before CB graft infusion. We have shown previously that with current dosing regimens with a fixed dose depicted as milligram per kilogram, higher post-HCT ATG exposures are found in higher body weight individuals and that small amounts of ATG severely hamper post-transplantation immune reconstitution [31]. Adult recipients are, therefore, more likely to have complete depletion of their CB graft using the haplo sCB regimens available. We hypothesize that in vivo T cell–depleted CB is less strong and more likely to not outcompete the T cell–depleted haplo graft, which is infused at the same time.

We confirm that in the context of sufficient serotherapy exposure, coinfusion of a $CD34^+$-selected haplo graft with CB transplantation can be a safe and effective treatment option even in a group of very high–risk patients (including patients with higher nonengraftment risks) with a low GVHD risk. As reported by others and observed in our cohort A, the majority of successfully engrafted patients switch to full CB chimerism within 4 months, followed by an ultimately normal immune recovery and repertoire [32]. However, this is delayed compared with the situation without serotherapy (exposure) in the conditioning regimen and completely dependent on thymic output after > 6 months after HCT [27,32,33]. Others have shown this successful CB engraftment in the haplo-cord setting regardless of the cell number of the CB, again arguing for a severe imbalance between the 2 grafts [11,34]. Most likely, the imbalance of a T cell–competent CB unit cotransplanted with a severely immune-depleted haplo unit (CD34 selection) facilitates maintenance of the CB unit. In double CB transplantations, dominant CB T cell clones have also been suggested to be involved in graft rejection of the subdominant unit [35,36].

Our observation has important implications for broadening the application of haplo-CB transplantations. Haplo-CB transplantations have been proposed as a method to use CB units with suboptimal cell numbers. This is in particular of interest for HLA-minority subgroups with a low probability of finding well-matched CB units [37] and for optimizing the use of highly matched, non inherited maternal antigens (NIMA) matched/mismatched CB or CB with specific genetic profiles [38,39]. Also, low ATG exposure may risk engraftment in (double) CB expansion protocols where the CD34-negative T cell–containing fraction of the expanded CB unit is not infused [40,41]. Furthermore, haplo-CB transplantation is increasingly being proposed as a cell support mechanism to drive engraftment with the goal of shortening hospital admission time and lowering costs [42,43]. Platelet engraftment is fast in haplo-CB studies, suggesting earlier transfusion independence [11,13]. Most interestingly, both neutrophil and platelet engraftment rates are faster than those reported in large double CB transplantation studies [44-46]. The advantage of the haplo donor here is the large number of stem cells that can be infused this way.

Haplo-CB protocols have been associated with low GVHD risks [11,13]. In our dataset, there is a striking difference between the low aGVHD incidence in patients treated with ATG (20%) and without ATG (75%, of which one-half were grade ≥ III). All patients with aGVHD had 100% CB chimerism at the time of aGVHD, suggesting the development of GVHD was not due to the alternative donor selection method used for the haplo donor. Instead, the nondepleted CB graft in the context without ATG is probably responsible for causing GVHD. This is consistent with our findings on the impact of Thymoglobulin in sCB transplantation, where much higher rates of GVHD were found in the patients who underwent transplantation without Thymoglobulin in the conditioning regimen [27].

Although limited by small sample size, omitting serotherapy (eg, ATG) in the haplo-CB setting appears to be strongly associated with failure of the haplo-myeloid-bridge to CB engraftment, resulting in high TRM rates and low survival. Being then confined to using ATG with a high post-HCT exposure, however, implies in vivo T depletion of the CB graft and resulting in slow T cell reconstitution, mostly dependent on thymic recovery [27]. Another strategy for patients with ongoing infection where an ultra-short neutropenic period in conjunction with a predictable early immune reconstitution is required could be bridging the time to

engraftment with granulocyte transfusions. A newer potentially interesting development is the development of CB expansion protocols that not only infuse the expanded stem cell population for early engraftment but also include the CD34-negative (T cell–containing) fraction in the graft to retain immune reconstitution capacity.

In adults, haplo-CB with serotherapy appears to be a good alternative to matched unrelated donors [47] with favorable outcomes even in elderly patients [48]. Being readily available, the haplo-CB platform has been proven useful when timing is of the essence and/or when only sCB and haplo donors are available. Haplo-CB can, therefore, specifically benefit patients without conventional donors and HCT patients with ongoing bacterial or fungal infection, but sufficient in vivo T depletion with ATG is required for its success.

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REFERENCES

1. Heemskerk MBA, van Walraven SM, Cornelissen JJ, et al. How to improve the search for an unrelated haematopoietic stem cell donor. Faster is better than more!. *Bone Marrow Transplant*. 2005;35:645-652.
2. Eapen M, Rubinstein P, Zhang M-J, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet*. 2007;369:1947-1954.
3. Willemze RR, Rodrigues CAC, Labopin MM, et al. KIR-ligand incompatibility in the graft-versus-host direction improves outcomes after umbilical cord blood transplantation for acute leukemia. *Leukemia*. 2009;23:492-500.
4. Kumar P, DeFor TE, Brunstein C, et al. Allogeneic hematopoietic stem cell transplantation in adult acute lymphocytic leukemia: impact of donor source on survival. *Biol Blood Marrow Transplant*. 2008;14:1394-1400.
5. Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. *N Engl J Med*. 1997;337:373-381.
6. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med*. 1998;339:1565-1577.
7. Parody R, Martino R, Rovira M, et al. Severe infections after unrelated donor allogeneic hematopoietic stem cell transplantation in adults: comparison of cord blood transplantation with peripheral blood and bone marrow transplantation. *Biol Blood Marrow Transplant*. 2006;12:734-748.
8. Törlén J, Ringdén O, Le Rademacher J, et al. Low CD34 dose is associated with poor survival after reduced-intensity conditioning allogeneic transplantation for acute myeloid leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2014;20:1418-1425.
9. Mateos MK, O'Brien TA, Oswald C, et al. Transplant-related mortality following allogeneic hematopoeitic stem cell transplantation for pediatric acute lymphoblastic leukemia: 25-year retrospective review. *Pediatr Blood Cancer*. 2013;60:1520-1527.
10. Klyuchnikov E, Asenova S, Kern W, et al. Post-transplant immune reconstitution after unrelated allogeneic stem cell transplant in patients with acute myeloid leukemia. *Leuk Lymphoma*. 2010;51:1450-1463.
11. Bautista G, Cabrera JR, Regidor C, et al. Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. *Bone Marrow Transplant*. 2008;43:365-373.
12. Martín-Donaire T, Rico M, Bautista G, et al. Immune reconstitution after cord blood transplants supported by coinfusion of mobilized hematopoietic stem cells from a third party donor. *Bone Marrow Transplant*. 2009;44:213-225.
13. Liu H, Rich ES, Godley L, et al. Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. *Blood*. 2011;118:6438-6445.
14. Gormley NJ, Wilder J, Khuu H, et al. Co-infusion of allogeneic cord blood with haploidentical CD34+ cells improved transplant outcome for patients with severe aplastic anemia undergoing cord blood transplantation. *Blood [ASH Annual Meeting Abstracts]*. 2011;118:654.
15. Hoegh-Petersen M, Amin MA, Liu Y, et al. Anti-thymocyte globulins capable of binding to T and B cells reduce graft-vs-host disease without increasing relapse. *Bone Marrow Transplant*. 2013;48:105-114.
16. Jakubowski AA, Small TN, Young JW, et al. T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin. *Blood*. 2007;110:4552-4559.
17. Luo X-H, Chang Y-J, Huang X-J. Improving cytomegalovirus-specific T cell reconstitution after haploidentical stem cell transplantation. *J Immunol Res*. 2014;2014:1-12.
18. Munchel AT, Kasamon YL, Fuchs EJ. Treatment of hematological malignancies with nonmyeloablative, HLA-haploidentical bone marrow transplantation and high dose, post-transplantation cyclophosphamide. *Best Pract Res Clin Haematol*. 2011;24:359-368.
19. Admiraal R, van Kesteren C, Zijde der CMJ-V, et al. Association between anti-thymocyte globulin (ATG) exposure and CD4+ immune reconstitution predicting overall survival in paediatric haematopoietic cell transplantation: a multicentre retrospective pharmacodynamic cohort analysis. *Lancet Haematol*. 2015;2:e194-e203.
20. Scheper W, van Dorp S, Kersting S, et al. γδT cells elicited by CMV reactivation after allo-SCT cross-recognize CMV and leukemia. *Leukemia*. 2013;27:1328-1338.
21. Scheper W, Gründer C, Straetemans T, et al. Hunting for clinical translation with innate-like immune cells and their receptors. *Leukemia*. 2014;28:1181-1190.
22. Zijde der CMCJ-V, Bredius RGMR, Jansen-Hoogendijk AMA, et al. IgG antibodies to ATG early after pediatric hematopoietic SCT increase the risk of acute GVHD. *Bone Marrow Transplant*. 2012;47:360-368.
23. Gerin M, Mahlaoui N, Elie C, et al. Therapeutic drug monitoring of voriconazole after intravenous administration in infants and children with primary immunodeficiency. *Ther Drug Monit*. 2011;33:464-466.
24. Chen J, Chan C, Colantonio D, Seto W. Therapeutic drug monitoring of voriconazole in children. *Ther Drug Monit*. 2012;34:77-84.
25. van Dorp S, Pietersma F, Wolfl M, et al. Rituximab treatment before reduced-intensity conditioning transplantation associates with a decreased incidence of extensive chronic GVHD. *Biol Blood Marrow Transplant*. 2009;15:671-678.
26. van Dorp S, Resemann H, Boome te L, et al. The immunological phenotype of rituximab-sensitive chronic graft-versus-host disease: a phase II study. *Haematologica*. 2011;96:1380-1384.
27. Lindemans CA, Chiesa R, Amrolia PJ, et al. Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood*. 2014;123:126-132.
28. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
29. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295-304.
30. Rebello P, Hale G. Pharmacokinetics of CAMPATH-1H: assay development and validation. *J Immunol Methods*. 2002;260:285-302.
31. Admiraal R, van Kesteren C, Jol-van der Zijde CM, et al. Population pharmacokinetic modeling of Thymoglobulin(®) in children receiving allogeneic-hematopoietic cell transplantation: towards improved survival through individualized dosing. *Clin Pharmacokinet*. 2015;54:435-446.
32. Jain N, Liu H, Artz AS, et al. Immune reconstitution after combined haploidentical and umbilical cord blood transplant. *Leuk Lymphoma*. 2013;54:1242-1249.

33. Kwon M, Bautista G, Balsalobre P, et al. Haplo-cord transplantation using CD34+ cells from a third-party donor to speed engraftment in high-risk patients with hematologic disorders. *Biol Blood Marrow Transplant*. 2014;20:2015-2022.
34. van Besien K, Liu H, Gergis U, et al. Haplo-cord UCB SCT with low cell dose, well matched UCB Units. A prospective multicenter study. *Blood*. 2014;124:1093.
35. Gutman JA, Riddell SR, McGoldrick S, Delaney C. Double unit cord blood transplantation: Who wins - and why do we care? *Chimerism*. 2010;1:21-22.
36. Gutman JA, Turtle CJ, Manley TJ, et al. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. *Blood*. 2010;115:757-765.
37. Barker JN, Byam CE, Kernan NA, et al. Availability of cord blood extends allogeneic hematopoietic stem cell transplant access to racial and ethnic minorities. *Biol Blood Marrow Transplant*. 2010;16:1541-1548.
38. Rocha V, Spellman S, Zhang M-J, et al. Effect of HLA-matching recipients to donor noninherited maternal antigens on outcomes after mismatched umbilical cord blood transplantation for hematologic malignancy. *Biol Blood Marrow Transplant*. 2012;18:1890-1896.
39. Eapen M, Klein JP, Ruggeri A, et al. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. *Blood*. 2014;123:133-140.
40. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med*. 2012;367:2305-2315.
41. Delaney C, Brashem-Stein C, Voorhies H, et al. Notch-mediated expansion of human cord blood progenitor cells results in rapid myeloid reconstitution in vivo following myeloablative cord blood transplantation. *Blood [ASH Annual Meeting Abstracts]*. 2008;112:212.
42. Barker JN, Ponce DM, Dahi P, et al. Double-unit cord blood (CB) transplantation combined with haplo-identical CD34+ cell-selected PBSC results In 100% CB engraftment with enhanced myeloid recovery. *Blood [ASH Annual Meeting Abstracts]*. 2013;122:298.
43. Sanchez ME, Ponce DM, Lauer E, et al. Double-unit cord blood (CB) transplantation (DCBT) combined with haplo-identical peripheral blood CD34+ cells (HaploCD34+) is associated with enhanced neutrophil recovery, universal haplo rejection, and frequent pre-engraftment syndrome. *Biol Blood Marrow Transplant [ASBMT Annual Meeting Abstracts]*. 2015;21:S43-S44.
44. Ponce DM, Zheng J, Gonzales A-M, et al. Reduced late mortality risk contributes to similar survival after double-unit cord blood transplantation compared with related and unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:1316-1326.
45. Kai S, Wake A, Okada M, et al. Double-unit cord blood transplantation after myeloablative conditioning for patients with hematologic malignancies: a multicenter phase II study in Japan. *Biol Blood Marrow Transplant*. 2013;19:812-819.
46. Wallet HL, Sobh M, Morisset S, et al. Double umbilical cord blood transplantation for hematological malignancies: A long-term analysis from the SFGM-TC registry. *Exp Hematol*. 2013;41:924-933.
47. Kwon M, Balsalobre P, Serrano D, et al. Single cord blood combined with HLA-mismatched third party donor cells: comparable results to matched unrelated donor transplantation in high-risk patients with hematologic disorders. *Biol Blood Marrow Transplant*. 2013;19:143-149.
48. Rhodes J, van Besien K, Liu H, et al. Haplo-cord Transplantation vs unrelated donor stem cell transplantation in patients with AML/MDS older than 50. *Blood [ASH Annual Meeting Abstracts]*. 2014;124:1235.

Use of Alefacept for Preconditioning in Multiply Transfused Pediatric Patients with Nonmalignant Diseases

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Transfusion-related alloimmunization is a potent barrier to the engraftment of allogeneic hematopoietic stem cells in patients with nonmalignant diseases (NMDs). Memory T cells, which drive alloimmunization, are relatively resistant to commonly used conditioning agents. Alefacept, a recombinant leukocyte function antigen-3/IgG1 fusion protein, targets CD2 and selectively depletes memory versus naive T cells. Three multiply transfused pediatric patients with NMD received a short course of high-dose i.v. alefacept (.25 mg/kg/dose on days −40 and −9 and .5 mg/kg/dose on days −33, −26, −19, and −12) before undergoing unrelated allogeneic transplant in the setting of reduced-intensity pretransplant conditioning and calcineurin inhibitor–based post-transplant graft-versus-host disease (GVHD) prophylaxis. Alefacept infusions were well tolerated in all patients. Peripheral blood flow cytometry was performed at baseline and during and after alefacept treatment. As expected, after the 5 weekly alefacept doses, each patient demonstrated selective loss of $CD2^{hi}/CCR7^{-}/CD45RA^{-}$ effector memory (Tem) and $CD2^{hi}/CCR7^{+}/CD45RA^{-}$ central memory (Tcm) $CD4^{+}$ and $CD8^{+}$ T cells with relative preservation of the $CD2^{lo}$ Tem and Tcm subpopulations. In addition, depletion of $CD2^{+}$ natural killer (NK) cells also occurred. Neutrophil recovery was rapid, and all 3 patients had 100% sorted (CD3/CD33) peripheral blood donor chimerism by day +100. Immune reconstitution (by absolute neutrophil, monocyte, and lymphocyte counts) was comparable with a cohort of historical control patients. All 3 patients developed GVHD but are all now off immune suppression and >2 years post-transplant with stable full-donor engraftment. These results suggest that alefacept at higher dosing can deplete both memory T cells and NK cells and that incorporating CD2-targeted depletion into a reduced-intensity transplant regimen is feasible and safe in heavily transfused patients.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many pediatric patients with life-threatening nonmalignant diseases (NMDs) [1-14]. Rejection remains an impediment to successful transplantation in many of these disorders, especially in

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