Published in final edited form as:

Transfusion. 2009 May ; 49(5): 995–1002. doi:10.1111/j.1537-2995.2008.02077.x.

High resolution HLA matching in Double Umbilical Cord Blood Reduced Intensity Transplantation (DCBT) in Adults

Meghan Delaney¹, Corey S. Cutler³, Richard L. Haspel⁴, Beow Y. Yeap², Steven L. McAfee², Bimalangshu R. Dey², Eyal Attar², Grace Kao³, Edwin P. Alyea³, John Koreth³, Vincent T. Ho³, Susan Saidman⁶, Deborah Liney³, Doreen Sese⁵, Robert J. Soiffer³, Thomas R Spitzer², Joseph H. Antin³, and Karen K Ballen²

¹Puget Sound Blood Center, Seattle, WA

²Hematology/Oncology Division, Department of Medicine, Massachusetts General Hospital, Boston, MA

³Medical Oncology/Hematological Malignancies, Dana Farber Cancer Institute, Boston, MA

⁴Pathology Department, Beth Israel Deaconess Medical Center, Boston, MA

⁵Tissue Typing Laboratory, Brigham and Women's Hospital, Boston, MA

⁶Histocompatibility Laboratory, Massachusetts General Hospital, Boston, MA

Abstract

BACKGROUND—Double cord blood transplantation (DCBT) offers an option for patients receiving reduced intensity transplants. These unique transplants have two donors, both of whom are usually HLA mismatched at 1–2 loci.

STUDY DESIGN AND METHODS—Forty-three patients were recipients of a reduced intensity DCBT. Cords were at least 4/6 allele level HLA-A, B, DR match with the patient and each other with minimum combined cell dose $>3.7 \times 10^7$ TNC/kg. Twenty-one patients received cyclosporine/mycophenolate mofetil (CYA/MMF) and 22 patients received sirolimus/tacrolimus (SIR/TAC) for GVHD prophylaxis. The effect of allele level HLA typing on clinical endpoints of overall survival (OS), disease-free survival (DFS), engraftment, and acute graft versus host disease (aGVHD) were assessed.

RESULTS—Neutrophil ($p=0.006$) and platelet ($p=0.033$) engraftment were significantly faster in patients who have closer HLA-B allele-level matching to their cord blood units. Within the CYA/MMF protocol, closer HLA-DR matching had a trend for less aGVHD: using both cords in the analysis 21% vs 57%, $p=0.053$, and the predominant cord alone 30% vs 57%, $p=0.143$. There was a low incidence of aGVHD in the SIR/TAC group and no difference in aGVHD incidence was associated with HLA-DR mismatching. HLA-A, C and DQ had no effect on engraftment or aGVHD.

CONCLUSION—HLA-DR matching was associated with a trend for lower risk of aGVHD in patients treated with CYA/MMF. HLA-B matching was associated with faster neutrophil engraftment and faster platelet engraftment. High resolution HLA matching did not affect OS, DFS, nor did it predict which cord will become predominant.

Corresponding author and reprint requests: Meghan Delaney, Puget Sound Blood Center, 921 Terry Avenue, Seattle, WA 98104, Phone: 206 233 3330, Fax: 206 343 1774, meghan@psbc.org.

The authors have no conflicts of interest.

SUMMARY/KEYWORDS

Transplantation; Stem Cell; double cord blood transplant (DCBT); umbilical cord blood (UCB); human leukocyte antigen (HLA); acute graft-versus-host disease (aGVHD); engraftment; predominant cord

INTRODUCTION

Unrelated double umbilical cord blood transplant (DCBT) is an acceptable alternative source for adult hematopoietic progenitor cell (HPC) transplantation in patients in whom a suitable adult source cannot be identified. An estimated 8000 single unit umbilical cord blood (UCB) transplants and over 300 DCBT have been completed since the first successful cord blood transplant was documented in 1989 by Gluckman et al.(1–3) The most fundamental obstacle to adult single donor UCB transplantation is the low cell dose causing an extended time to engraftment and increased treatment related mortality. (4–10) Double cord blood transplantation may ameliorate an extended engraftment period by allowing more stem cells to survive and repopulate the marrow compartment. (11–13)

In unrelated bone marrow or peripheral blood stem cell transplantation, high resolution class I (HLA-A, -B, -C) and class II (HLA-DRB1, -DQ) human leukocyte antigen (HLA) matching is crucial. (14–15) Petersdorf and colleagues showed in the unrelated setting that allele-level mismatching for class I alleles (HLA-A, B, C) can cause increased graft failure and that class II (HLA-DRB1, DQ) mismatches are associated with aGVHD. (15) A recent NMDP study of high resolution HLA typing of >3800 unrelated adult donor transplants (94% bone marrow, 6% peripheral blood) found that one locus mismatch (7/8) of HLA-A, B, C or DR compared to no allele mismatches (8/8) was associated with lower survival, higher treatment related mortality, and more aGVHD. (16) While most HLA matching for UCB has been done at the allele level for MHC class II, antigen level typing is the norm for class I, and 1 – 2 mismatches are typically acceptable. Mismatching at HLA-C is also not usually considered in cord blood donor searches, although it has been shown to influence the likelihood of graft failure, increase GVH and decrease survival in unrelated peripheral blood stem cell transplantation in chronic myelogenous leukemia (CML) patients. (17–18)

Few data exist on the effect of locus specific HLA matching in cord blood transplantation and matching beyond HLA-A, B and DR. As we gain experience, the importance of HLA disparities, originally thought to be relatively unimportant in UCB transplantation, has become more compelling. (19) For example, Kogler and colleagues found a higher than expected degree of allele level HLA disparity in a large cohort of UCB transplant recipients when they retrospectively typed them using high-resolution methods that had not been available at the time of transplantation. (20) HLA-A,-B and -DR analysis by antigen level typing found a trend for worse two year survival with poorly matched grafts; for example, patients with a 6/6 matched graft had a 2-year survival of 45% compared to 13% for 3/6 matched grafts (p=0.38). High resolution analysis (HLA-A, -B, -C, -DRB1, -DQB1) yielded similar 2-year survival findings; 10/10 53%, 9/10 32%, 8/10 47%, 7/10 38%, 6/10 41%, 2-5/10 39% (p=0.29). Causes of death in this series were infection (24) relapse (22), other (17) and GVHD (10). The authors argued that there was little need for high resolution typing based on these data because they did not find a correlation between number of high resolution HLA mismatches and 2-year survival. Furthermore, although a low cumulative incidence of acute graft versus host disease (aGVHD) was seen overall, a trend of HLA-B mismatching in those that did develop grade II – IV aGVHD was observed. (20–21) Other researchers have since found correlation between HLA antigen mismatches and aGVHD in single UCB transplantation. Specifically, the presence of both class I and II HLA disparity was associated with grades III-IV aGVHD,

$p=0.0093$. (22) A recently published review described the current studies on HLA matching and UCB and found that there was increasing evidence that HLA does play a role in survival and hematopoietic recovery. (19)

Because double cord blood transplants are unique in that they have two donors, both of whom are usually HLA mismatched (MM) at 1 – 2 loci and only antigen-level matched at class I loci, the degree of HLA disparity between donors and recipient can be great. Few programs use allele level typing for their double cord blood transplant patients. Therefore, we inquired whether compatibility determined by high-resolution typing at HLA-A, B, C, DR, DQ influences overall survival (OS), disease-free survival (DFS), engraftment, and acute graft versus host disease (aGVHD).

MATERIALS AND METHODS

Patients

Patients were eligible for reduced intensity DBCT transplant if they had no 5/6 or 6/6 HLA-A, B, DR matched related donor or no 6/6 HLA matched A, B, DR unrelated donor available. Patients were between the ages of 18–65 years and met institutional transplant eligibility criteria including ejection fraction greater than 45% and lung diffusion capacity (DLCO) greater than 50% predicted. Patients with acute leukemia were eligible if they were in 2nd or subsequent complete remission or in first remission with high-risk cytogenetics. Patients with relapsed non-Hodgkin's lymphoma, Hodgkin's disease and chronic lymphocytic leukemia progressive after at least two chemotherapy regimens were also eligible. Aplastic anemia and myelodysplasia with any IPSS score were eligible. Refractory leukemia or leukemia in relapse were not eligible. Conditioning regimen was fludarabine 30 mg/m²/day on days -8 through -3 (total dose 180 mg/m²), melphalan 100 mg/m²/day on day -2, and rabbit antithymocyte globulin (Genzyme, Cambridge, MA) 1.5 mg/kg/days on days -7, -5, -3, -1 (total dose 6.0 mg/kg). Patients were entered into one of two sequential protocols that differed only in respect to GVH prophylaxis. Twenty-one patients received cyclosporine continuous intravenous infusion starting on day -3 and mycophenolate mofetil 15 mg/kg intravenously twice daily starting on day 0 (CYA/MMF), with tapering of MMF after Day +60 and CYA after Day +100. (13) Twenty two patients received sirolimus (trough serum concentration 3 – 12ng/mL) and tacrolimus (trough serum concentration 5 – 10ng/mL) (SIR/TAC) for GVHD prophylaxis. (23) The protocols were reviewed and approved by the Institutional Review Board of the Dana Farber/Harvard Cancer Center. Written informed consent was obtained from all patients prior to participation.

HLA Typing and Cord Unit Selection

Patient HLA typing was done with high resolution molecular typing for HLA-A, -B, -C, -DR and -DQ by the polymerase chain reaction and sequence specific primer method (Invitrogen Corporation, Brown Deer, WI) or restriction fragment length polymorphisms (New England Biolabs, Ipswich, MA) using thermal cyclers (ABI 2720, 9600, 9700, Applied Biosystems, Foster City, CA). Cord unit high resolution HLA typing was carried out by the respective donor centers, and confirmed at our transplant center. Only HLA-A, -B, and -DR were considered in matching for transplantation. Cords were 4/6 or higher allele level HLA-A,-B, DR match with the patient and each other.

Cord units were obtained from national and international registries. Cord units were selected to favor higher cell dose; if two units had similar HLA matching, the larger unit was chosen. Combined cord blood units met a minimum pre-freeze combined cell dose of $>3.7 \times 10^7$ TNC/kg. After accruing 15 patients, the protocol was amended so the minimum cell dose for each individual cord was $>1.5 \times 10^7$ TNC/kg. Cord units were washed with dextran and albumin

and were infused sequentially 1 to 6 hours apart. (24) HLA matching interpretation was considered in both graft-versus-host (GvH), and host-versus-graft (HvG) directions. However, due to small sample size, the analysis of these subsets precluded meaningful interpretation, therefore a mismatch was identified using either direction of mismatch.

Endpoints

We studied HLA matching of the patient to both donor cord units combined, to the predominant cord, and between the two cords (irrespective of patient HLA type) for the following endpoints: overall survival (OS), disease-free survival (DFS), engraftment, and acute graft versus host disease (aGVHD). The predominant cord was defined as the cord that contributes >60% peripheral blood leukocytes at ninety days. This group has previously reported that the majority of patients will have one predominant cord within this time period. (25) Chimerism assays of peripheral blood were used to determine the cord predominance and were performed by short tandem repeat (STR) analysis using a multiplex kit with primers for 10 different loci (Profiler Plus, Applied Biosystems, Foster City, CA) and an ABI-310 and ABI-3130 (after January 2006) Genetic Analyzers (Applied Biosystems, Foster City, CA). (13,23,25) Neutrophil engraftment was defined as an absolute neutrophil count (ANC) of greater than 500 cells/ μ L for three consecutive days. Platelet engraftment was defined as the first day with an unsupported platelet count of >20,000/ μ L and no platelet transfusion within 7 days.

Biostatistical Analysis

All engraftment and survival times were measured beginning from Day 0 of cord blood infusion. In order to increase the power for exploratory analysis, the cord blood groupings based on HLA matching were chosen so that the patient numbers in comparison groups were distributed as equally as possible. Times to ANC and platelet engraftment were based on the cumulative incidence estimated in the presence of death, relapse and graft loss as competing risks. Patients who had not engrafted or failed due to a competing risk were censored at the last follow-up date their blood counts were checked. The relationship between engraftment time and HLA matching was analyzed using the Gray test to compare cumulative incidence curves between groups. (15) In addition, the engraftment analysis was stratified by defining two subgroups based on the median TNC or CD34 dose of the whole patient group as a threshold. The cumulative incidence of aGVHD $\geq$ grade 2 was estimated by considering death and graft loss as competing risks. Patients who had not experienced aGVHD $\geq$ grade 2, graft loss or death were censored at their last follow-up. The Gray test comparing aGVHD incidence was stratified by protocol in order to control for differences due to GVHD prophylaxis. In order to examine whether the effect of HLA matching were influenced by age, the aGVHD analysis was stratified further according to patient subgroups ≤ 50 and > 50 years old. McNemar's test was used to examine the relationship between HLA matching and cord unit predominance, while the logrank test was used to assess differences in OS and DFS. OS was measured until the date of death or was censored at the last follow-up for patients who were still alive. DFS was measured until the earlier of relapse or death, while it was censored at the last follow-up for patients who were still alive free of relapse. The *cmprsk* package in R version 2.5.1 was used in the analysis of competing risks, while SAS 9.1 was used to perform the remaining computations. All p-values are based on two-sided hypothesis tests.

RESULTS

Patient and Cord Characteristics

Forty-three consecutive patients, aged 19 to 64 years (median 49 years) were recipients of a reduced intensity DCBT. Diseases treated were acute leukemia, non-Hodgkin's lymphoma, Hodgkin's disease, chronic lymphocytic lymphoma, myelodysplastic syndrome and other hematologic disease, see Table 1. The racial composition consisted of 36 (84%) Caucasians,

3 Hispanics, 2 African-Americans, 1 Asian and 1 recipient of unidentified race. Thirty (70%) patients received two 4/6 matched cords, 9 (21%) received one 4/6 and one 5/6 and 4 (9%) received two 5/6 cords, see Table 2a and 2b. No patients received two completely matched cord blood units. The median CD34+ cell counts/kg was 1.9 median (0.3–9.7 range) $\times 10^5$ and the median nucleated cell count/kg was 4.2 median (2.9–7.5 range) $\times 10^7$.

As reported previously within this group, the CYA/MMF group had overall and disease-free survivals of 71% and 67% at one year, respectively. (13) Within the SIR/TAC group, overall and disease-free survivals were 73% and 51% at one year, respectively. (23)

Combined Cords

Combined cords (both cords together) HLA typing was compared to the patient's HLA type (Table 2a).

For Class I typing, six alleles (HLA-A, -B, -C) from each donor were considered, thus a perfect match in this instance would be 12/12. There were no 12/12 matches. A trend for improved time to ANC $>500/\mu\text{L}$ was detected with increased class I alleles (HLA-A, -B, -C) matching; median of 20.5 days (7-10/12 match) versus 24 days (5-6/12 match, $p=0.077$). This trend was not seen with platelet engraftment and class I alleles; median time to engraftment was 43.5 versus 42 days, respectively ($p=0.629$). Class I matching had no effect on aGVHD.

We studied the HLA-A, B and C loci of the cord units compared to the patient's HLA type; a perfect match was 4/4 for each locus. Neutrophil engraftment was faster in patients who had closer HLA-B matching to their combined cords (median of 19 versus 22 days, $p=0.006$, Table 3 and Figure 1a). There was a trend to faster platelet engraftment in patients who had better HLA-B matching with their combined cords (median of 42 versus 50 days, $p=0.033$, Table 3 and Figure 1c). Eight patients were 4/4 HLA-B match; they engrafted neutrophils and platelets at a median of 19.5 days ($p=0.063$) and 40.5 days ($p=0.012$), respectively. HLA-A and C did not demonstrate similar findings.

Similarly, combined class II (HLA-DR, -DQ) loci typing were analyzed together in relation to the recipient. A perfect match in this instance is 8/8. Closer HLA class II matching (6-8/8 versus 4-5/8) was associated with a lower 100-day incidence of aGVHD, grade II – IV (18% versus 57%, respectively, $p=0.015$). When each Class II loci (HLA-DR or DQ) was studied separately, patients with 4/4 HLA-DR match had 20% 100-day incidence of aGVHD, which supports the finding that closer HLA-DR matching is associated with lower incidence of aGVHD. This difference appears to be primarily due to HLA-DR matching in the CYA/MMF subjects. Within the CYA/MMF group, but not in the SIR/TAC group, patients with better HLA-DR matching (3-4/4 versus 2/4) to combined cords had less aGVHD (100-day incidence of 21% versus 57%, $p=0.053$, Table 4 and Figure 2a). This finding is particularly intriguing, given that high resolution matching for HLA-DR is known to be important for aGVHD in other transplant settings. Class II matching had no effect on engraftment.

There were few patients with chronic GVHD; 8 total patients had cGVHD, 3 had extensive cGVHD, thus a formal statistical analysis was not performed.

Predominant Cord

We undertook further study of the HLA disparity and clinical endpoints using the predominant cord alone, defined as the cord that contributes $>60\%$ peripheral blood leukocytes at ninety days (Table 2b). (25) Patients who were better matched to the predominant cord at grouped class I HLA alleles had minimally faster neutrophil engraftment (4-6/6, median 19.5 days) than those that were not as well matched (2-3/6, median 21 days, $p=0.027$).

Individual HLA loci analysis found that the ten patients who were fully matched at HLA-B (2/2) with their predominant cord engrafted neutrophils faster (median 17.5 days) compared to those that had one mismatch (median 21 days, $p=0.035$, Table 3 and Figure 1b). A similar trend was observed with HLA-B when compatibility between the patient and the predominant cord was assessed for platelet engraftment (median of 41.5 versus 45.5 days, $p=0.124$, Table 3 and Figure 1d). There were no patients with 0/2 HLA-B match with their predominant cord.

Although the patient population was small, close HLA class II matching in the predominant cord (3-4/4 versus 2/4) showed a trend for lower 100-day incidence of aGVHD (23% versus 43%, respectively, $p=0.135$). The association of aGVHD and class II matching correlated mostly with CYA/MMF patients and not SIR/TAC patients in the HLA-DR locus. HLA-DR matching of the patient to predominant cord in the CYA/MMF protocol showed a trend to less aGVHD (100-day incidence of 30% versus 57%, $p=0.143$, Table 4 and Figure 2b), but the power of the study is limited by the small patient numbers. HLA matching did not affect overall or disease-free survival, or which cord blood unit eventually predominated.

Cord to Cord

Double cord blood transplant offers the opportunity to compare HLA matching between two donors; a perfect match would be 10/10. The times to neutrophil engraftment and platelet engraftment were not affected by HLA matching between the two cord blood units. The 100-day incidence of aGVHD was 15% in 7-10/10 versus 56% in 5-6/10 ($p=0.042$). The rate of graft failure was not affected by HLA matching between the two cord blood units, including the HLA-DR locus ($p=0.412$). However, the power is limited by only 3 patients who experienced early graft loss; days 35, day 36 and at day 102.

Stratified analysis

The trends detected for engraftment with respect to HLA-B matching were not affected by the infused cell dose. The results in Table 3 for combined cords and predominant cord retained their statistical significance when the analysis was controlled for infused TNC or CD34 dose. The only exception was the association between time to ANC engraftment and HLA-B matching in the predominant cord was diminished when stratified by CD34 dose ($p=0.164$). Among the patients infused with a lower CD34 dose (defined as $\leq$ median in the whole group), those fully matched at HLA-B (2/2) with their predominant cord engrafted neutrophils faster (median 16 days) compared to those who had one mismatch (median 22 days, $p=0.004$).

The association between aGVHD incidence and HLA-DR matching within the CYA/MMF group as shown in Table 4 was not influenced by age stratification. The consistency was observed for combined cords, predominant cord and cord-cord results.

DISCUSSION

Several groups have shown that DBCT offers a viable option for adults in need of reduced intensity hematopoietic stem cell transplantation who do not have a fully matched related or unrelated donor. (11,13,23) High-resolution HLA-matching has proven to be important in unrelated bone marrow and peripheral blood stem cell transplants and was studied here in double cord blood transplants. (15,26)

We found a statistically significant association between class I matching to the cord blood units with faster neutrophil engraftment and less robust evidence for faster platelet engraftment. Furthermore, patients whose cord units or predominant cord matched at HLA-B appeared to engraft faster than those patients that had mismatching at HLA-B. A biological hypothesis for this finding may be because HLA-B is one of the targets for natural killer cells inhibitory

receptor (KIR). (27–28) Since host natural killer cells may persist in the non-myeloablative setting, a mismatch at HLA-B could allow NK cells to selectively kill recently infused donor cells and cause prolonged engraftment, although our data did not detect a difference with HLA-Cw (another KIR ligand) and engraftment. KIR matching was not studied in our analysis. Prospective typing at the KIR locus could contribute information in the future.

The clinical implications for our findings is in donor unit selection: choosing two units with close HLA-B match to the patient may shorten the neutropenic period and perhaps lessen the time the patient is at risk for fungal and other opportunistic infections. However, our study design did not permit a direct comparison between intermediate and high resolution HLA matching at Class I. Investigators at the University of Minnesota have shown that antigen level matching for Class I HLA alleles in non-myeloablative cord blood transplants gives favorable results. (29) It is logical to propose, that the favorable results found with allele level matching in marrow transplantation might extend to cord blood transplantation. Our series of patients also demonstrates good overall outcomes and, much like Kogler's analysis, finds trends and some statistically significant differences for the role of HLA in clinical outcomes, but does not support the notion that allele level mismatching negatively impacts overall survival. The added time and cost of high resolution HLA typing for class I alleles may not justify the expense and delay in this setting, as HLA matching had no effect on overall survival.

We also found class II HLA matching of the combined cords to the recipient correlated with grades II-IV aGVHD, although our overall incidence of aGVHD was low. This finding was driven by HLA-DR, and is not surprising, given that high resolution matching for HLA-DR is known to be associated with aGVHD in other transplant settings. (26) Because the incidence of aGVHD was three-fold higher in patients with HLA-DR mismatches with their combined cords, and two-fold higher in patients with HLA-DR mismatching in their predominant cord, we propose that there may be less aGVHD in patients that receive cords matched at HLA-DR. This study also shows that with improved GVHD prophylaxis regimens, such as sirolimus and tacrolimus, HLA matching may become less important. (25)

Limitations of the current study are attributable to the small sample size and relatively short follow-up time. Moreover, many other factors affect clinical outcomes in cord blood transplantation, such as age, cell dose, disease and disease status. Our study also employed two GVH regimens, which yielded different aGVHD results with similar survival. Lastly, because our cord donors and patient HLA typing was undertaken using high resolution methods, we are limited in our ability to determine if low resolution would have been sufficient for HLA-matching. The potential benefit of reduced engraftment time must be balanced against the added cost of high resolution typing if our findings are used to guide unit selection and pre-transplant cord unit confirmatory HLA typing.

Creating an algorithm of HLA matching for umbilical cord blood unit selection in double unit transplantation, combined with the known beneficial effect of choosing units with higher cell doses, may be a valuable approach to improve clinical endpoints. (30) We have shown that high resolution matching for HLA-B may predict faster neutrophil engraftment and HLA-DR may predict a lower incidence of aGVHD. These findings are applicable for guiding cord blood donor registry searches.

ACKNOWLEDGEMENTS

We would like to thank Dr Karen Nelson, PhD for her critical review of this manuscript.

Source of support: NIH grant HL070149

REFERENCES

1. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA identical sibling. *N Engl J Med* 1989 Oct 26;321(17):1178-8
2. Ballen KK, Barker JN, Stewart SK, Greene MF, Lane TA. Collection and preservation of cord blood for personal use. *Biol Blood Marrow Transplant* 2008 Mar;14(3):356-363. [PubMed: 18275904]
3. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci USA* 1989 May;86:3828-3832.
4. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, Verfaillie CM, Wagner JE. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 2005 Feb;105:1343-1347. [PubMed: 15466923]
5. Ballen KK. New trends in umbilical cord blood transplantation. *Blood* 2005;105:3786-3792. [PubMed: 15677563]
6. Gluckman E. Hematopoietic stem-cell transplants using umbilical-cord blood. *N Engl J Med* 2001 Jun 14;344(24):1860-1861. [PubMed: 11407350]
7. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, Goldman A, Kersey J, Krivit W, MacMillan ML, Orchard PJ, Peters C, Weisdorf DJ, Ramsay NK, Davies SM. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases; influences of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood* 2002 Sept 1;100(5):1611-1618. [PubMed: 12176879]
8. Styczynski J, Cheung YK, Garvin J, Savage DG, Billote GB, Harrison L, Skerrett D, Wolownik K, Wischhover C, Hawks R, Bradley MB, Del Toro G, George D, Yamashiro D, van de Ven C, Cairo MS. Outcomes of unrelated cord blood transplantation in pediatric recipients. *Bone Marrow Transplantation* 2002;34:129-136. [PubMed: 15107815]
9. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, Berkowitz RL, Cabbad M, Dobrila NL, Taylor PE, Rosenfield RE, Stevens CE. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 1998 Nov 26;339(22):1565-1577. [PubMed: 9828244]
10. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, Marks DI, van Rood JJ, Scaradavou A, Horowitz MM. Outcomes after Transplantation of Cord Blood or Bone Marrow from Unrelated Donors in Adults with Leukemia. *N Engl J Med* 2004;351:2265-2275. [PubMed: 15564543]
11. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood* 2003;102:1915-1919. [PubMed: 12738676]
12. Barker JN, Scaradavou A, Stevens CE, Rubinstein P. Analysis of 608 umbilical cord blood (UCB) transplants: HLA-match is a critical determinant of transplant-related mortality (TRM) in the post-engraftment period even in the absence of acute graft-versus-host disease (aGVHD). *American Society of Hematology Annual Meeting Abstracts* 2005;106:303.
13. Ballen KK, Spitzer TR, Yeap BY, McAfee S, Dey BR, Attar E, Haspel R, Kao G, Liney D, Alyea E, Lee S, Cutler C, Ho V, Soiffer R, Antin JH. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol Blood Marrow Transplant* 2007 Jan;13(1):82-89. [PubMed: 17222756]
14. Petersdorf EW, Malkki M. Human Leukocyte Antigen Matching in Unrelated Donor Hematopoietic Cell Transplantation. *Seminars in Hematol* 2005;42:76-84.
15. Petersdorf EW, Gooley TA, Anasetti C, Martin PJ, Smith AG, Mickelson EM, Woolfrey AE, Hansen JA. Optimizing Outcome After Unrelated Marrow Transplantation by Comprehensive Matching of HLA Class I and II Alleles in the Donor and Recipient. *Blood* 1998;92(10):3515-3520. [PubMed: 9808542]
16. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, Fernandez-Vina M, Flomenberg N, Horowitz M, Hurley CK, Noreen H, Oudshoorn M, Petersdorf E, Setterholm M, Spellman S, Weisdorf D, Williams TM, Anasetti C. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007 Sep 4;

17. Petersdorf EW, Anasetti C, Martin PJ, Gooley T, Radich J, Malkki M, Woolfrey A, Smith A, Mickelson E, Hanson JA. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. *Blood* 2004;104(9):2976–2980. [PubMed: 15251989]
18. Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M, Hurley C, Kollman C, Anasetti C, Noreen H, Begovich A, Hildebrand W, Petersdorf E, Schmeckpeper B, Setterholm M, Trachtenberg E, Williams T, Yunis E, Weisdorf D. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood* 2004;104(7):1923–1930. [PubMed: 15191952]
19. Kamani N, Spellman S, Hurley CK, Barker JN, Smith FO, Oudshoorn M, Bray R, Smith A, Williams TM, Logan B, Eapen M, Anasetti C, Setterholm M, Confer DL. State of the art review: HLA matching and outcome of unrelated donor umbilical cord blood transplants. *Biol Blood Marrow Transplant* 2008;14:1–6. [PubMed: 18158954]
20. Kögler G, Enczmann J, Rocha V, Gluckman E, Wernet P. High-resolution HLA typing by sequencing for HLA-A, -B, -C, -DR, -DQ in 122 unrelated cord blood/patient pair transplants hardly improves long-term clinical outcome. *Bone Marrow Transp* 2005;36:1033–1041.
21. Gluckman E, Koegler G, Rocha V. Human Leukocyte Antigen Matching in Cord Blood Transplantation. *Semin Hematol* 2005;42:85–90. [PubMed: 15846574]
22. Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan KW, Takahashi TA, Ortega J, Filipovich A, Locatelli F, Asano S, Fagioli F, Vowels M, Sirvent A, Laporte JP, Tiedemann K, Amadori S, Abecassis M, Bordigoni P, Diez B, Shaw PJ, Vora A, Caniglia M, Garnier F, Ionescu I, Garcia J, Koegler G, Rebulla P, Chevret S. Eurocord Group. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol* 2004 Apr;32(4):397–407. [PubMed: 15050751]
23. Cutler C, Mitrovich R, Kao G, Ho V, Alyea E, Koreth J, Armand P, Dey B, Spitzer T, Soiffer R, Antin JH, Ballen K. Double Umbilical Cord Blood Transplantation with Reduced Intensity Conditioning and Sirolimus-Based GVHD Prophylaxis. *Blood* 2007 Nov;118(11)Abstract 2016, 600a
24. Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci U S A* 1995;92:10119–10122. [PubMed: 7479737]
25. Haspel RL, Kao G, Yeap BY, Cutler C, Soiffer RJ, Alyea EP, Ho VT, Koreth J, Dey BR, McAfee SL, Attar EC, Spitzer T, Antin JH, Ballen KK. Preinfusion variables predict the predominant unit in the setting of reduced-intensity double cord blood transplantation. *Bone Marrow Transplant* 2008 Mar;41(6):523–529. [PubMed: 18037942](in press)
26. Petersdorf EW, Kollman C, Hurley CK, Dupont B, Nademanee A, Begovich AB, Weisdorf D, McGlave P. Effect of HLA class II gene disparity on clinical outcome in unrelated hematopoietic cell transplantation for chronic myeloid leukemia: the US National Marrow Donor Program Experience. *Blood* 2001;98(10):2922–2929. [PubMed: 11698272]
27. De Santis D, Bishara A, Witt CS, Nagler A, Brautbar C, Slavin S, Christiansen FT. Natural Killer cell HLA-C epitopes and killer cell immunoglobulin-like receptors both influence outcome of mismatched unrelated bone marrow transplants. *Tissue Antigens* 2005 Jun;65(6):519–528. [PubMed: 15896199]
28. Denis L, Sivula J, Gourraud PA, Kerdudou N, Chout R, Ricard C, Moisan JP, Gagne K, Partanen J, Bignon JD. Genetic diversity of KIR natural killer cell markers in populations from France, Guadeloupe, Finland, Senegal and Reunion. *Tissue Antigens* 2005 Oct;66(4):267–276. [PubMed: 16185321]
29. Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, McGlave PB, Wagner JE. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. *Blood* 2007 Oct 15;110(8):3064–3070. [PubMed: 17569820]
30. Barker JN, Scaradavou A, Stevens C, Rubinstein P. The Dose-Match Interaction in Umbilical Cord Blood (UCB) Transplantation: An Analysis of the Impact of Cell Dose and HLA-Match on the Disease Free Survival (DFS) of 989 Patients Transplanted with Single Units for Hematological Malignancy. *Blood* 2007;110(11):333.144a

Figure 1. HLA-B Mismatching and Engraftment

Engraftment curves grouped by high resolution HLA-B matching. a) Combined cords and neutrophil engraftment, $p=0.006$, b) Predominant cord and neutrophil engraftment, $p=0.035$, c) Combined cords and platelet engraftment, $p=0.033$, d) Predominant cord and platelet engraftment, $p=0.124$.

Figure 2. HLA-DR Matching and acute GVHD

Incidence of acute graft-versus-host disease grade 2–4 grouped by high resolution HLA-DR matching. a) Combined cords in CYA/MMF treatment group, $p=0.053$, b) Predominant cord in CYA/MMF treatment group, $p=0.143$, c) Combined cords in SIR/TAC treatment group, $p=0.749$, d) Predominant cord in SIR/TAC treatment group, $p=0.965$.

Table 1

Patient demographics

	CYA/MMF	SIR/TAC
Age, range (median)	49 years (24–63)	52.5 years (19–64)
Male	15 (71%)	11 (50%)
Non-Caucasian	4	2
AML: CR1 high risk	4	4
AML: CR2 or greater	4	2
ALL	1	0
NHL	5	8
HD	2	3
CLL	2	1
MDS	1	3
Other	2	1

Table 2

Table 2a: Recipient/Combined Cord Donors HLA Matching		
HLA Loci		Combined Cords No. patients (%) High Resolution N = 43
A	4/4	18 (42%)
	3/4	4 (9%)
	2/4	21 (49%)
	1/4	0
	0/4	0
B	4/4	8 (19%)
	3/4	3 (7%)
	2/4	32 (74%)
	1/4	0
	0/4	0
C	4/4	5 (12%)
	3/4	3 (7%)
	2/4	20 (47%)
	1/4	9 (21%)
	0/4	2 (5%)
	Not tested	4 (9%)
DR	4/4	20 (47%)
	3/4	3 (7%)
	2/4	20 (47%)
	1/4	0
	0/b4	0
DQ	4/4	19 (44%)
	3/4	5 (12%)
	2/4	16 (37%)
	1/4	0
	0/4	0
	Not tested	3 (7%)

Table 2b: Recipient/Predominant Cord Donors HLA Matching		
HLA Loci		Predominant Cord Number Pts (%) High Resolution N = 38*
A	2/2	17 (45%)
	1/2	21 (55%)
	0/2	0
B	2/2	10 (26%)
	1/2	28 (74%)
	0/2	0
C	2/2	7 (18%)
	1/2	24 (63%)
	0/2	5 (13%)
	Not tested	2 (5%)
DR	2/2	17 (45%)
	1/2	21 (55%)
	0/2	0
DQ	2/2	19 (50%)
	1/2	18 (47%)
	0/2	0
	Not tested	1 (3%)

* 5 patients had no predominant cord detected

Table 3

HLA-B Matching and Engraftment

HLA-B Matching	Combined Cords		Predominant Cord	
	3-4/4	2/4	2/2	1/2
Median time to ANC >500 (days)	19	22 p=0.006	17.5	21 p=0.035
Median time to Platelets >20K (days)	42	50 p=0.033	41.5	45.5 p=0.124

Table 4HLA-DR Matching and aGVHD $\geq$ grade 2

HLA-DR Matching	Combined Cords		Predominant Cord	
	3-4/4	2/4	2/2	1/2
aGVHD Grades II-IV	17%	30% p=0.077	24%	29% p=0.209
CYA/MMF protocol	21%	57% p=0.053	30%	57% p=0.143
SIR/TAC protocol	11%	15% p=0.749	14%	14% p=0.965