State of the Art Review: HLA Matching and Outcome of Unrelated Donor Umbilical Cord Blood Transplants

Naynesh Kamani,1 Stephen Spellman,2 Carolyn Katovich Hurley,3 Juliet N. Barker,4 Franklin O. Smith,5 Machteld Oudshoorn,6 Robert Bray,7 Anajane Smith,8 Thomas M. Williams,9 Brent Logan,10 Mary Eapen,10 Claudio Anasetti,11 Michelle Setterholm,2 Dennis L. Confer2

1 Children’s National Medical Center, Washington, DC; 2 National Marrow Donor Program, Minneapolis, Minnesota; 3 Department of Oncology, Georgetown University, Washington, DC; 4 Memorial Sloan-Kettering Cancer Center, New York, New York; 5 Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, Ohio;6 Europdonor Foundation, Leiden, The Netherlands; 7 Emory University School of Medicine, Atlanta, Georgia; 8 Seattle Cancer Care Alliance, Seattle, Washington; 9 University of New Mexico School of Medicine, Albuquerque, New Mexico; 10 CIBMTR Statistical Center, Medical College of Wisconsin, Milwaukee, Wisconsin; and 11H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Correspondence and reprint requests: Dennis Confer, MD, National Marrow Donor Program, 3001 Broadway Street NE, Minneapolis, MN 55413 (e-mail: dconfer@nmdp.org).

Received October 31, 2007; accepted November 10, 2007

INTRODUCTION

This commentary, sponsored by the National Marrow Donor Program® (NMDP), reviews the current, relevant data concerning the importance of HLA matching in umbilical cord blood (UCB) transplantation (UCBT). Our goal is to provide guidance for UCB unit selection and to identify the information that is needed to refine the minimum and optimal matching guidelines.

Background

The successful use of partially HLA-mismatched unrelated donor UCB as a source of hematopoietic stem cells (HSCs) was first reported by Kurtzberg et al [1] in 1996. In the subsequent decade, several studies have shown that the results of HSC transplants, predominately in pediatric patients, using HLA matched or partially mismatched unrelated donor UCB, are comparable to those using unrelated donor bone marrow [2-6]. UCB is now accepted as an alternative source of HSCs for unrelated donor transplantation. There are currently over 47 public UCB banks around the world that have a combined inventory of over 260,000 UCB units (marrow.org and BMDW). Over 8000 UCB transplants have been carried out worldwide, and UCB is being increasingly utilized as a source for HSC [7].

Analyses of unrelated donor bone marrow transplant (UBMT) outcomes have shown an impact of HLA matching on outcomes such as hematopoietic engraftment, graft-versus-host disease (GVHD), and overall survival (OS). The following document summarizes current data and provides guidance in selecting UCB units for unrelated donor UCBT based on HLA matching. This commentary extends previous guidelines from the NMDP on optimal donor-recipient matching for unrelated marrow transplants [8] to include selection of unrelated donor UCB units. Because much of the data on the impact of HLA match on UCBT is currently preliminary or inconclusive, the NMDP will continue to monitor the literature and research developments in the field to amend and refine guidelines over time.

Assessment of HLA Match Status in UCBT Donor-Recipient Pairs

When reviewing the cord blood transplant literature, it is important to note that the majority of UCBT’s reported have utilized matching at the antigen level for HLA-A and HLA-B loci and at the allele level for HLA-DRB1 loci. HLA-A and -B were typed either by serology or low- to intermediate-resolution DNA-based methods. In the first report of UCBT, prospective typing of UCB units for the DRB1 locus at the allele level was carried out for UCB unit selection.
when more than 1 unit mismatched at 2 loci was available [1]. This approach was likely based on an early recognition of the importance of DRB1 allele matching in UCBT [9]. Donor-recipient matching was thus categorized into 6/6, 5/6, 4/6, and greater mismatching based on this level of resolution.

Recent studies in UBM have identified the importance of allelic disparity between donor and recipient [10-13] in determining transplant outcomes. Although HLA matching at low to intermediate resolution for HLA-A and -B and allele level matching for DRB1 continues to be the current standard for cord blood unit selection, a number of recent retrospective analyses have evaluated the impact of the undetected blood unit selection, a number of recent retrospective studies, and is a limitation in determining the current role of HLA matching in UBM studies.

Impact of HLA Matching on Engraftment after UCBT

Hematopoietic engraftment after UCBT differs from that seen following either UBM or peripheral blood stem cell transplantation in 2 important respects. There is a significant delay in the time to engraftment (as measured by the time to recovery of an absolute neutrophil count of >500/μL or time to achievement of a platelet count of 20-50,000/μL independent of transfusion support), and the overall probability of engraftment is lower compared to other stem cell sources [2,3,5,6,18,21]. Studies of UCBT have consistently shown that the cell dose is strongly correlated with hematopoietic engraftment. Although the total nucleated cell dose is a critical factor impacting engraftment [22-24], additional studies have suggested that the CD34+ cell dose [25] or the graft progenitor cell content as measured by colony-forming cells [26] may be more important determinants of hematopoietic recovery post-UCBT. However, as the quantification of CD34+ cells or colony-forming cells is difficult to standardize from bank to bank, the nucleated cell dose is more widely accepted as the criterion by which UCB units are selected.

In addition to graft cell dose, analyses of outcomes following UCBT have suggested an impact of donor-recipient HLA matching on engraftment. Although this was not seen in a smaller single institution study of 102 recipients [25], in the largest analysis done to date on 562 UCBT recipients, Rubinstein et al [22] have shown that there is a progressive delay in myeloid engraftment by day 42 with increasing HLA mismatching. In their analysis, 100% of patients receiving HLA-A, -B, and -DRB1 matched transplants engrafted (n = 40) compared to 78% (95% confidence interval [CI], 72%-85%) of 211 recipients of 5/6 matched UCBT, 82% (95% CI, 76%-88%) of 257 recipients of 4/6 matched UCBT, and 69% (95% CI, 52-86%) of 39 patients receiving grafts mismatched at 3 or more loci (P = .01). The largest number of patients received grafts that were 1 or 2 antigen mismatched, but the authors could not detect a significant difference in myeloid engraftment between those who received grafts mismatched at 1 or 2 antigens. In univariate analysis, platelet engraftment to a count of 50,000/microliter was not associated with the extent of HLA disparity [22]. In the COBLT study, multivariate analysis showed that a higher original HLA match (5 or 6/6) correlated with neutrophil recovery (P = .04) [18]. However, when the retrospective HLA match was considered this correlation could not be shown (P = .19).

The interaction of cell dose with HLA was not evaluated in the COBLT study, but it has been analyzed in a recent study by Eapen et al [2] comparing outcomes of 503 UCBTs and 282 UBM Ts in children...
<16 years with acute leukemia to assess the influence of both cell dose and HLA matching on outcome. A cutoff for cell dose for 1 antigen mismatched transplants was defined as $3 \times 10^7$/kg. Eapen et al [2] found that the probability of neutrophil recovery by day 42 and platelet recovery by 6 months was similar after marrow or matched UCBT but lower for mismatched UCBT at both low and high cell doses compared to UBMT. Higher cell doses resulted in a higher probability of both neutrophil and platelet recovery in 1-antigen-mismatched UCBT but had no effect in 2-antigen mismatched transplants, suggesting that cell dose may not be able to overcome the adverse impact of mismatching in the setting of 4 out of 6 matched UCBT.

An impact of HLA matching upon engraftment has also been demonstrated by the the European Blood and Marrow Transplant Group. In an analysis of 550 UCBTs, they found that the 60-day cumulative incidence of neutrophil engraftment for all patients was 74%, whereas the incidence for those with no HLA disparities versus ≥3 disparities were 83% and 53.2%, respectively ($P = .001$). The number of HLA disparities was correlated with neutrophil recovery with a log-linear relationship between HLA disparity and risk of graft failure, suggesting inferior engraftment with increased disparity. Only 263 of 550 patients (50.5%) achieved an untransfused platelet count of 20,000/μL by day 180, with an absence of both HLA-A, -B, and -DRB1 disparities being correlated with a higher 180-day cumulative incidence of platelet recovery ($P = .006$). The EBMT study was unable to demonstrate a statistically significant interaction between the cell dose and HLA disparities for either neutrophil or platelet recovery [27].

In an analysis of the pooled datasets of the New York Placental Blood Program, the National Marrow Donor Program, and the COBLT Study, Gibbons analyzed the effects of cell dose and HLA mismatch on engraftment after UCBT and found that patients receiving HLA mismatched UCBT had a significantly higher graft failure rate ($P < .001$) than those receiving 6/6 matched cords ($P < .002$ and $P < .007$, respectively, for patients with 4/6 and 5/6 mismatched UCBT) [28]. In summary, whereas cell dose is important for engraftment in UCBT, HLA match also appears to impact the engraftment rates. Table 1 summarizes the findings from the larger studies that evaluated the impact of HLA match (antigen-level HLA-A, -B, and allele-level HLA-DRB1) on engraftment.

### Impact of HLA Matching on GVHD

Several analyses of UCBT have assessed the impact of HLA matching on GVHD incidence. The analysis of the National Cord Blood Program of the New York Blood Center’s experience showed that there was a trend suggesting an impact of matching on risk of severe grade III-IV GVHD with recipients of matched UCB showing a lower GVHD risk compared to recipients of mismatched UCB ($P = .06$). There was no impact of matching on chronic GVHD (cGVHD) among recipients of mismatched UCB [22]. The analysis of Eurocord data shows that whereas the degree of matching did not impact overall GVHD risk, the risk of grade III-IV GVHD was higher in recipients of UCB where class I and II disparities coexisted between donor and recipient [27]. In the COBLT study, multivariate analysis of the impact of the original HLA matching (intermediate resolution at class I and allele level at class II) on incidence of acute GVHD (aGVHD) showed that HLA matching impacted grade II-IV risk with a significantly higher risk of aGVHD in recipients of 4/6 matched UCB compared to 5-6/6 matched UCB ($P = .03$) [18]. The outcome comparison study of UBM versus UCB by Eapen et al [2] showed a significantly higher risk of both aGVHD and cGVHD with the use of matched BM compared to UCB, but failed to show a correlation between HLA match and aGVHD or cGVHD risk among recipients of UCB transplants. Table 2 summarizes the

### Table 1. HLA Match: Effect of Mismatching on Engraftment (Defined as Achieving ANC ≥ 500)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6</td>
<td>100%</td>
<td>83%</td>
<td>85%</td>
<td>Favorable ($P = .04$)</td>
</tr>
<tr>
<td>5/6</td>
<td>78% (NS)</td>
<td>Decrease (NS)</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>4/6</td>
<td>82% (NS)</td>
<td>Decrease (NS)</td>
<td>76%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>≥3/6</td>
<td>69% ($P &lt; .01$)</td>
<td>53.2% ($P &lt; .01$)</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*45-day evaluation.
†60-day evaluation.
‡42 day evaluation.
§Multivariate analysis results comparing ≥5/6 to ≤4/6; hazard ratio 1.39 95%CI (1.02-1.89), $P = .04$.

### Table 2. HLA Match: Effect of Mismatching on GVHD

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute GVHD</td>
<td>6/6 match increases grade III-IV risk ($P = .06$)</td>
<td>Increased risk of grade III-IV with class I and class II mismatch</td>
<td>No impact</td>
<td>&lt;5/6 match increases grade II-IV risk ($P = .03$)</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>No impact</td>
<td>Not evaluated</td>
<td>No impact</td>
<td>Not evaluated</td>
</tr>
</tbody>
</table>
findings from the larger studies that evaluated the impact of HLA match (antigen-level HLA-A, B, and allele-level HLA-DRB1) on GVHD and the findings from studies that evaluated the impact of HLA match on aGVHD and/or cGVHD.

**Role of HLA Compatibility on Survival after UCBT**

Only a few reports of UCBT have analyzed the effects of HLA mismatching on patient survival. Rubinstein et al [22], and later Wagner et al [25], reported the association of HLA-mismatching with the outcome of UCBT. Both reports identified the detrimental effects of HLA-A, -B, and -DRB1 mismatching on survival. Rubinstein et al [22] found that event-free survival (EFS) (with event defined by death, autologous reconstitution, or second transplant) was associated with HLA incompatibility ($P = .004$ in univariate analysis). A multivariable Cox model analysis found a higher risk of these adverse events in 217 UCBTs with 1 mismatch (relative risk [RR] = 2.0, 95% CI 1.1-3.6), and 300 UCBTs with 2 or more mismatches (RR = 2.5, 95% CI 1.4-4.5) compared with 40 matched cord blood transplants. Although the power of the study was limited, there was no significant effect of the number of mismatched loci, type of mismatched locus, and level of typing resolution utilized to define the mismatch at DRB1. Wagner et al [25] found an increased risk of death (RR = 2.4, 95% CI 1.2-4.7) among 44 recipients of UCBTs mismatched for 2-3 loci, compared to 58 mismatched for 0-1 locus (multivariable $P = .01$). Eapen et al [2] evaluated treatment failure as the inverse of leukemia-free survival (LFS) and showed a favorable outcome for matched UCBT compared to 1 to 2 antigen mismatched UCBT or matched or allele-mismatched UBMT ($P = .041$). It is to be noted that there were only 35 patients in the matched UCB cohort, but suggests that HLA matching may be of strong import in outcome after UCBT.

In contrast to the reports by Rubinstein et al [22] and Wagner et al [25], a study of 550 UCBTs by Eurocord [27] failed to find an effect of HLA disparity on 3-year survival with estimates of 34% with an HLA-A, -B, -DRB1 match (n = 53), 38% with 1 mismatch (n = 243), 32% with 2 mismatches (n = 218), and 33% with 3 or 4 mismatches (n = 36) ($P = .30$). The reasons why these results were discordant with those from the Rubinstein et al [22] and Wagner et al [25] studies are not obvious; however, cell dose did not enter the multivariable model in the Eurocord study, whereas it was significant and was retained in the multivariable models of both the Rubinstein et al [22] and Wagner et al [25] studies. It should be noted that in the Eurocord analysis of patients with malignant diseases, increasing HLA disparity resulted in lower rates of engraftment and higher treatment-related mortality (TRM) and cGVHD, but this did not translate into a lower disease-free (DFS) or OS because of a lower relapse rate. HLA disparity did impact survival for patients with nonmalignant diseases [7].

It has been proposed that a higher graft cell dose is required for engraftment and survival after mismatched than after matched UCBTs. TRM was significantly lower among patients who received 1 antigen mismatched UCB and a relatively high cell dose (>30 million total nucleated cells [TNCs] per kg), whereas those who received 1 antigen mismatched UCB and a lower cell dose or recipients of 2 antigen mismatched UCB regardless of cell dose had higher TRM. This effect did not translate into a higher LFS for recipients of higher cell dose 1 antigen mismatched UCBT [2]. Because the effects of HLA-mismatching and cell dose appear to interact, it is possible that the potential effect of HLA may be masked if cell dose is not considered in the analysis. Table 3 summarizes the findings of the largest studies to date that evaluated the role of HLA match on survival following UCBT.

The impact of high-resolution HLA matching in UCBT has been investigated in a number of papers. Ohnuma et al [16] reported that mismatching for 2-4 HLA-A, B, or DRB1 alleles (n = 13) was associated with decreased survival (35% versus 75%) compared to mismatching for 0-1 alleles (n = 14) ($P = .02$). Cornetta et al [17] reported no effect of HLA-mismatching at low resolution on the 1-year survival in a small study of 24 adults. When high-resolution typing was used to define HLA-A, -B, and -DRB1 alleles, 3 of 4 patients with 0-1 mismatches survived >1 year compared to 2 of 20 patients with 2 or 3 mismatches. Both these analyses suggest that high-resolution HLA typing may help select UCB units better suitable for a favorable outcome.

Kogler et al [15] retrospectively typed 122 UCBT donor and recipient pairs by high resolution at

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5/6</td>
<td>Decrease</td>
<td>Decrease</td>
<td>No effect †</td>
<td>Decrease</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>≤ 4/6</td>
<td>Decrease</td>
<td>Decrease</td>
<td>No effect †</td>
<td>Decrease</td>
<td></td>
</tr>
</tbody>
</table>

†Cell dose was not considered in the multivariate analysis.

‡Leukemia free survival.
HLA-A, -B, -C, -DRB1, and -DQB1, but failed to show any association with survival in univariate analyses. A multivariable model including cell dose as covariate was not shown in the paper, and therefore the potential confounding interaction of cell dose with HLA-mismatching might have masked a possible association of HLA mismatching with survival. In the COBLT study, multivariable analysis using retrospective high-resolution typing data showed that there was a significant increase in the incidence of grades II-IV and III-IV acute GVHD (P = .02) and a significant decrease in survival probability when a 2–4/6 matched UCBT was compared to 5–6/6 matched transplants (P = .04) [18].

When interpreting all the analyses described above, the following limitations need to be kept in mind. First, only a small proportion of patients have received fully matched UCB transplants, and most of them have been pediatric patients. Second, the majority of transplants performed to date have been 1 or 2 antigen mismatched, and larger patient numbers may be required to fully determine the differences between these 2 cohorts. Third, although there are suggestions of an interaction between cell dose and HLA matching, the sample sizes thus far have limited the investigation of this interaction. In addition, patient age and cell dose may be confounding variables.

Other Considerations in UCB Unit Selection

Although the availability of adequately dosed and HLA-matched UCB units is important, there are a number of other factors that need to be considered when selecting a unit for transplant. A number of additional factors may influence how a transplant physician chooses UCB units including: the CB bank, whether the unit was red cell depleted prior to cryopreservation, and availability of attached segments for confirmatory typing and infectious disease marker characteristics of the unit. There are no data currently available, however, that have evaluated the role of these in engraftment or survival after UCB transplantation.

SUMMARY

UCB graft cell dose is a critical determinant of hematopoietic recovery and survival after UCBT. However, there is increasing evidence that HLA match is also a key factor in UCBT outcome with mismatch adversely impacting on both engraftment and survival. Because of the relatively low numbers of patients transplanted with UCB to date it is not currently known how cell dose and HLA match interact as determinants of UCBT outcome. Also, it is not clear whether the same criteria will apply to both pediatric and adult recipients. Current data suggest that HLA match is critically important in the setting of a low cell dose. The elucidation of the impact of cell dose and match on UCBT outcome will be a major research priority for the future, and may impact determinations of the size of the cord blood inventory. Specifically, the available data does not allow us to fully discern the impact of a 1 versus 2 antigen mismatch, or how to “trade-off” the HLA-match and cell dose in unit selection. In addition, the importance of allele level matching at HLA-A and B, the match vector, and whether HLA-C or DQB1 should be considered in the selection of UCB units for transplantation is yet to be determined, and will rely on the collection of the appropriate allele level data to permit future analyses. The interaction between cell dose and HLA matching is especially critical in adults undergoing UCBT, and underlines the need for drastic increases in the unrelated cord blood inventory. Table 4 summarizes the NMDP’s current recommendations for typing and matching of UCB units for transplantation based on the current literature. All recommendations are based on selection of a unit with an appropriate cell dose. Currently available data would consider this to be a unit that has $>2.5-3 \times 10^7$ total precryopreserved nucleated cells per kg recipient body weight. It is hoped, however, that an increased UCB inventory will allow patients to receive units that are both of better match and sufficient cell dose, and that this will result in improved hematopoietic engraftment and survival after UCBT.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Resolution of Typing for UCB Confirmatory Testing</th>
<th>Resolution of Match for UCB transplant‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Allele level*</td>
<td>Antigen level</td>
</tr>
<tr>
<td>B</td>
<td>Allele level*</td>
<td>Antigen level</td>
</tr>
<tr>
<td>C</td>
<td>Allele level*</td>
<td>Unknown†</td>
</tr>
<tr>
<td>DRB1</td>
<td>Allele level</td>
<td>Allele level</td>
</tr>
<tr>
<td>DQB1</td>
<td>Allele level*</td>
<td>Unknown†</td>
</tr>
</tbody>
</table>

*Recommend testing for HLA-C and DQB1 to facilitate retrospective analyses.
†Unknown indicates that the effect of matching is inconclusive.
‡Four of 6 matching at HLA-A, -B, -DRB1 for release of CBU for transplantation.

ACKNOWLEDGMENTS

The authors would like to thank Dr. John Wagner for a critical review of the manuscript.

REFERENCES


