Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation

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Short Title: Anti-HLA Antibodies in DUCBT
Abstract

Using a uniform detection method for donor specific anti-HLA antibodies (DSA), we sought to determine the effect of preformed DSA on outcomes in double umbilical cord blood transplantation. DSA were associated with an increased incidence of graft failure (5.5% vs. 18.2% vs. 57.1% for none, single or dual DSA positivity, p = 0.0001), prolongation of the time to neutrophil engraftment (21 vs. 29 days for none vs. any DSA, p=0.04), and excess 100-day mortality or relapse (23.6% vs. 36.4% vs. 71.4% for none, single or dual DSA positivity, p=0.01). The intensity of DSA reactivity was correlated with graft failure (median of mean fluorescent intensity 17650 vs. 1850, p=0.039). There was inferior long-term progression-free and overall survival when comparing patients with DSA against both umbilical cord blood units to those without DSA (3 year PFS: 0% vs. 33.5%, p = 0.004; 3 year OS 0% vs. 45.0%, p=0.04). We conclude that identification of preformed DSA in umbilical cord blood recipients should be performed and that the use of umbilical cord blood units where preformed host DSA exist should be avoided.
Introduction

In North America, the use of double umbilical cord blood (UCB) transplantation has largely supplanted single UCB transplantation in adults due to a more reliable and shorter time to neutrophil engraftment. However, prediction of the dominantly engrafting umbilical cord blood unit after double UCB transplantation is an inexact science. Factors such as total nucleated cell dose, CD34+ cell dose, HLA match and order of UCB unit infusion have logically been associated with engraftment, however, none of these factors reliably predict the dominant engrafting unit.1-3

Anti-HLA antibodies may be observed in healthy individuals,4,5 however in patients with hematological diseases, anti-HLA antibodies are more frequently detected due to the frequent use of transfusion therapy and alloimmunization.6 In a small series, Gutman et al estimated the incidence of preformed anti-HLA antibodies to be 9% among individuals being considered for UCB transplantation.7 In solid organ transplantation, where crossing HLA barriers is routine, anti-HLA antibodies are linked to organ rejection and inferior outcomes.8,9 In hematopoietic stem cell transplantation, more limited data exist on the significance of these antibodies. Spellman et al, in a retrospective case-control study, demonstrated that the prevalence of donor-specific anti-HLA antibodies (DSA) was higher in a group of mismatched unrelated donor recipients who suffered graft rejection than in a control group that engrafted.10 This analysis does not allow an accurate estimate of the impact of DSA in individual patients due to the case-control design. In umbilical cord blood transplantation, case reports have demonstrated that engraftment after single UCB transplantation can occur even in the presence of DSA,11,12 however, Takanashi et al have recently demonstrated a significant reduction in the cumulative incidence of neutrophil engraftment and inferior outcomes in the presence of DSA after myeloablative single UCB transplantation.13,14

By examining all double UCB transplants at our institution, using a single standardized methodology for the detection of DSA, we sought to determine if the presence of DSA had a detrimental effect on outcomes after double UCB transplantation.
Methods

**Umbilical Cord Blood Transplantation**

All subjects underwent transplantation using sequentially administered double UCB units after either myeloablative or reduced intensity conditioning. Myeloablative conditioning consisted of fludarabine, cyclophosphamide and total body irradiation (TBI) while reduced intensity conditioning consisted of fludarabine, melphalan and anti-thymocyte globulin (ATG). GVHD prophylaxis was with cyclosporine and mycophenolate mofetil or tacrolimus and sirolimus. UCB products were administered between 1 and 6 hours apart. The unit with the higher total nucleated cell (TNC) count was administered first. All patients have been previously reported.\(^{15,16}\)

UCB units had a minimum combined pre-cryopreservation cell dose of \(3.7 \times 10^7\) TNC/kg and each individual unit was required to have a minimum of \(1.5 \times 10^7\) TNC/kg prior to cryopreservation. UCB units were required to be a 4/6 match or better at the allele level for HLA-A, -B, and -DR\(\beta\)1 with each other and with the recipient. The choice of UCB units, when multiple units were available, was hierarchically based on a higher cell dose, greater HLA compatibility and a younger age of the cord blood unit. The presence of DSA was not routinely considered in cord blood selection, however DSA status might have been known to the treating physician.

**Solid phase screening methodology to detect anti-HLA antibodies**

All analyses were performed on cryopreserved pre-transplantation patient serum, which was examined for the presence of anti-HLA antibodies against HLA-A, B, C, DR and DQ. Cord units were not HLA typed at HLA-DP and therefore this antigen was not considered. For general screening for anti-HLA antibodies, LABScreen Mixed microbeads (One Lambda Inc., Canoga Park, CA) coated with purified class I or class II HLA antigens were used. 5µl of microbeads were incubated with 20µl of pre-transplantation patient serum for 30 minutes then washed eight times. 100µl of anti-human phycoerythrin were added to each well. After 30 minutes and three washes, the assay was run on the Luminex100 IS System instrument (Luminex Corp., Austin, TX) to
detect fluorescent tagged binding of human IgG. To determine the presence/absence of class I and II antibodies, results used the normalized ratio calculated by Visual software. Positive screening samples were subsequently tested for anti-HLA antibodies against individual class I and II antigens. Assays with 1000 mean fluorescent intensity (MFI) above baseline were considered positive for defining the presence of anti-HLA antibodies. Anti-HLA antibodies were only considered for analysis if they were directed against Class I or II HLA specificities found in the transplanted cord blood units.

**Statistical Analysis**

Patient baseline characteristics and UCB unit characteristics were reported descriptively. Fisher’s exact test or Wilcoxon-rank-sum test were used for two-group comparisons. The Cochran-Armitage test was used to test a trend in proportions of events (graft failure, early death or relapse) for multiple group comparisons. Neutrophil engraftment was defined as the first of three consecutive days with neutrophil recovery to at least 0.5 x 10⁹ cells/L with umbilical cord blood hematopoiesis measured by short-tandem repeat chimerism assessment. Platelet engraftment was defined as the first day of a platelet count of 20 x 10⁹/L, without supporting transfusion in the prior 3 days. Graft failure was defined as the absence of neutrophil engraftment by day 42 from double UCB transplantation or loss of UCB chimerism by day 100 without malignant relapse. Time to engraftment was calculated reflecting death or relapse without engraftment as a competing risk. Malignancy relapse risk was defined by standard CIBMTR criteria.

Cumulative incidence curves for acute and chronic GVHD were constructed reflecting death or relapse as competing risks. Cumulative incidence curves for non-relapse mortality (NRM) and relapse with or without death were constructed reflecting time to relapse and time to NRM as competing risks. Time to relapse and time to NRM were measured from the date of stem cell infusion. Overall survival (OS) was defined as the time from transplantation to death from any cause, while progression-free survival (PFS) was defined as the time from transplantation to progression or death from any cause. Surviving patients were censored at their date of last known follow-up. The log-rank test was used for comparisons of Kaplan-Meier curves; Gray’s test was used for
comparisons of cumulative incidence curves. Potential prognostic factors for OS, PFS, relapse, NRM, and engraftment were examined in proportional hazards models as well as in competing risks regression models\textsuperscript{18}. Due to the small number of graft failures, potential prognostic factors for graft failure were examined using univariable exact logistic regression analysis. To explore whether DSA intensity, measured as MFI, predicted graft failure, an analysis of receiver operator characteristics (ROC) was performed. All p-values are two-sided. Significance was defined at p=0.05 level. All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC) and R 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).
Results

We analyzed 73 patients who underwent double UCB transplantation between 2004 and 2008. DSA were detected in 18 patients: 9 patients had DSA directed against the first infused UCB unit, 2 patients had DSA directed against the second infused UCB unit, and 7 patients had DSA directed against both UCB units. Four patients had multiple DSA directed against cord blood units. Baseline characteristics of these patients are shown in Table 1, UCB graft characteristics are shown in Table 2 and the specificity and intensity of the DSA with engraftment outcomes are shown in Table 3. During the time period examined, an additional 26 cord blood transplants were performed. Reasons for not being included in this analysis include: second transplantation(7), single umbilical cord blood transplantation(1), death prior to stem cell administration (1) and lack of banked serum (17). Of the 17 patients with no samples for analysis, 14 patients successfully engrafted while 3 patients expired prior to day 30 without engraftment.

Graft Failure

A total of 9 patients (12.3%) suffered from graft failure. The incidence of graft failure in patients without DSA, with DSA directed against a single UCB unit (n=11), or DSA directed against both UCB units (n=7) was 5.5%, 18.2% and 57.1% respectively (Cochran-Armitage trend test p = 0.0001) (Figure 1). Individual odds ratios (OR) for graft failure comparing patients with DSA against one UCB unit, DSA against one or both UCB units, or DSA against both UCB units to patients without DSA were 3.85 (95% CI 0.56 – 26.4, p= 0.19), 8.67 (95% CI 1.89 – 39.68, p= 0.0055) and 23.1 (95% CI 3.5 – 153.9, p= 0.002) respectively. These statistical associations were maintained when patients who underwent reduced intensity conditioning (n=53) were examined. The incidence of graft failure in reduced intensity patients without DSA (n=40), with DSA directed against a single UCB unit (n=7), or DSA directed against both UCB units (n=6) was 2.5%, 14% and 67% respectively (Cochran-Armitage trend test p < 0.001). Overall, 6 of 18 patients with any DSA experienced graft failure. It is notable that 3 of 4 patients with DSA directed against multiple HLA antigens on a single cord blood unit experienced graft failure.
Also notable is that among the 11 patients with a single DSA (9 against unit 1, 2 against unit 2), 3 failed to engraft, and an additional patient died by day 100, leaving 7 patients evaluable for day 100 chimerism and engraftment analysis. Of these 7 patients, 2 engrafted with the unit against which there was only low level DSA (MFI 1100, 1200), 3 engrafted with the unit without DSA (MFIs against the non-engrafting unit: 5500, 7500, 18000) and 2 had prolonged (greater than 1 year) persistence of both units (intermediate MFIs: 1800, 2300). Only 1 subject with DSA against both units had persistence of a graft at day 100. The MFI in this case was 1200 and was directed against a common HLA-B antigen shared by both cord units.

Univariable exact logistic regression analysis identified only the presence of a DSA as a significant predictor of graft failure (Exact OR 8.32, 95% CI 1.53 – 58.88, p= 0.01). Non-significant factors tested in this model included recipient age and sex, donor/recipient sex mismatch, malignancy risk status, myeloid vs. lymphoid malignancy, prior stem cell transplantation, the use of sirolimus as GVHD prophylaxis and CD34+ and TNC doses infused. Multivariable regression analysis could not be performed due to the small number of events.

The intensity of DSA measured by MFI correlated with the occurrence of graft failure. The median MFI among 12 DSA patients without graft failure was 1850 whereas it was 17650 in the 6 DSA patients who experienced graft failure (p=0.039). Five of 6 DSA patients with MFI values greater than 7 000 experienced graft failure in comparison with 1 of 12 patients with values below 7 000 (positive predictive value 83%, sensitivity 83%, AUC 0.81).

**Time to Engraftment**

The median time to engraftment of neutrophils was 23 days (range 13 – 70 days) in the entire cohort. When stratified by the presence or absence of DSA, the median time to neutrophil engraftment was 21 days without DSA, and 29 days in the presence of a DSA (p=0.04) (Figure 2). There was no difference in the time to engraftment in patients with DSA against a single versus both UCB units (30 vs. 27 days, p = NS).
The median time to an unsupported platelet count was 42 days for the entire cohort. In patients with any DSA the median time to platelet engraftment was 50 days, in comparison with 42 days in patients without DSA (p=1.0). In patients without DSA, the cumulative incidence of platelet engraftment by day 180 was 75%. The corresponding figures for patients with DSA against one or both UCB units were 64% and 29% (p = 0.23), however it is worth noting that the majority of patients with DSA died early after transplantation.

In multivariable competing risk regression analyses examining the impact of DSA on the cumulative incidence of neutrophil engraftment, the presence of any DSA (p=0.01) or DSA against both UCB units (p=0.015) were associated with a reduction in the cumulative incidence of engraftment, while factors such as age, donor/recipient sex mismatch, conditioning intensity, year of transplant, malignancy risk or myeloid vs. lymphoid malignancy were not statistically associated with engraftment. When either CD34+ dose or TNC dose were included in the models, the presence of any DSA retained statistical significance (p=0.019 and 0.013 respectively). When CD34+ dose was included in the models examining the impact of DSA against both UCB units, there was a trend associating DSA and engraftment (p=0.053), while the association remained statistically significant when TNC was included in the model (p=0.016). Neither CD34+ nor TNC dose independently attained statistical significance in any of the models.

**GVHD, Early Mortality and Long-Term Survival**

The cumulative incidence of grade II-IV acute GVHD for the entire cohort was 17.8%. However, none of the patients with DSA developed acute GVHD, while the cumulative incidence of acute GVHD in the group without DSA was 23.6% (p=0.025). This result is related to the small number of patients in the DSA group who were at risk of acute GVHD, since 14 of these 18 patients either relapsed or died within 100 days of transplant. No differences in the rates of chronic GVHD were noted in patients surviving beyond day 100 (23.3% overall).
Similar to graft failure, death or relapse within 100 days of transplantation was correlated with the presence of DSA. The rates of death or relapse within 100 days for the group of patients without DSA, with DSA against a single UCB unit or DSA against both UCB units were 23.6%, 36.4% and 71.4% (Cochran-Armitage trend test p=0.01) (Figure 3). Relapse with or without death within 100 days of transplantation was significantly associated with DSA status (14.5%, 18.2% and 57.1%, Cochran-Armitage trend test p=0.017) however non-relapse mortality within 100 days of transplantation was not significantly different between no DSA and DSA patients (9% vs. 17%, p=NS). Causes of death among patients with DSA included sepsis/infection (4), relapse (3), VOD (1), GVHD (1), and other (1).

Univariable exact logistic regression analysis identified only the presence of DSA against both UCB units as a significant predictor of early death or relapse (Exact OR 6.98, 95% CI 1.03 – 79.69, p= 0.046). The presence of DSA against a single UCB unit approached statistical significance (Exact OR 3.17, 95% CI 0.91 – 11.31, p=0.07). Non-significant factors in this model included recipient age and sex, donor/recipient sex mismatch, malignancy risk status, myeloid vs. lymphoid malignancy, prior stem cell transplantation, the use of sirolimus as GVHD prophylaxis and CD34+ and TNC doses infused. In multivariable exact logistic regression analysis, the presence of DSA against both UCB units retained statistical significance for early death or relapse (p = 0.03). In a parallel multivariable model, there was a trend associating the presence of DSA against a single UCB unit and early death or relapse (p=0.08). When TNC or CD34+ dose were added to these models, the presence of DSA against both UCB units retained statistical significance (p=0.012 when TNC added, p=0.021 when CD34+ added). When either TNC or CD34+ cell count were added to the model examining the influence of only a single DSA, the presence of DSA was predictive of early death or relapse (p=0.039 for TNC, p=0.029 for CD34+). CD34+ cell count or TNC were not independently predictive of early death or relapse in any model construct.

Progression-free (PFS) and overall survival (OS) at 3 years from transplantation were 30% (95% CI 19.5% – 41.4%) and 43.3% (30.6% – 55.4%) for the entire cohort, with a
median follow-up among survivors of 33.8 months. Three year PFS and OS were non-significantly shorter in patients with any DSA when compared to those without DSA (PFS: 18.5% vs. 33.5%, p=0.14; OS: 40.0% vs. 44.8%, p=0.81). However, when comparing patients with DSA directed against both UCB units with those without any DSA, both PFS and OS were significantly shorter (3 year PFS: 0% vs. 33.5%, p = 0.004; 3 year OS 0% vs. 45.0%, p=0.04) (Figures 4a, 4b). When examined in a Cox proportional hazards model, the presence of DSA against both UCB units and malignancy risk status were both significantly correlated with both PFS and OS (p<0.05 for all outcomes). The presence of a single DSA itself did not attain statistical significance whereas malignancy risk was correlated with both PFS (p=0.05) and OS (p=0.06) in a parallel model.
Discussion

In this retrospective analysis, we have demonstrated that the presence of preformed DSA directed at transplanted UCB units can have important clinical consequences. In patients with preformed DSA against transplanted UCB units, there is an increased incidence of graft failure, a delay in the time to neutrophil and platelet engraftment, excess early mortality and decreased long-term overall survival. These findings demonstrate the necessity to screen for DSA prior to the selection of UCB units for eventual transplantation.

The mechanism of DSA mediated cord blood rejection is unknown. In solid organ transplantation, the presence of donor specific antibodies (either against HLA or other antigens) leads to the deposition of terminal complement factors in the transplanted tissue and is associated with chronic allograft injury and dysfunction. The specific role of T cells in this process is unknown, however, in allogeneic stem cell graft rejection, it is likely that T cells play an important role. If T cells are involved, then determining the origin of these T cells is of critical importance.

It is difficult to elucidate the individual roles of residual host and donor T cells in the rejection process, since processes such as in vivo T cell depletion, associated with increased rates of graft failure, simultaneously depletes both host and donor T cells. Ex vivo T cell depletion, which removes donor T cells only, is also associated with increased rates of graft failure, arguing against the role of donor T cells in graft failure. While the number of T cells delivered with the graft is low, it is clear that these T cells are immunologically active. Recently, Gutman et al have identified an UCB vs. UCB CD8+ T cell response that predicted UCB dominance after double UCB transplantation in 9 of 10 patients examined. Whether pre-formed DSA primed the T cell response in these cases is unknown. In addition, other cellular mediators, such as NK cells, may be involved. One factor relevant to our analysis is that over 70% of our patients underwent RIC conditioning, which includes ATG, and functionally depletes both recipient and
some amount of donor T cells. Since the majority of patients underwent RIC transplantation, the influence of ATG use could not be tested in the multivariable models. In addition, since the presence of DSA may have been known to the treating physician, this could have influenced unit selection, and the overall incidence of graft failure.

If preformed DSA are pathogenic, then a strategy to circumvent the ill effects of these antibodies should be sought. Unfortunately, strategies to reduce the production of antibody, either through depletion of B cells (with monoclonal antibodies such as rituximab) or depletion of plasma cells (with proteosome inhibitors such as bortezomib), are unlikely to be successful unless the continued production of DSA is implicated in the graft rejection process. If complement mediated pathways are involved in stem cell graft rejection, then strategies that include the use of the terminal complement inhibitor eculizumab could be useful, however evidence supporting the role of complement activation in stem cell graft rejection is lacking. In solid organ transplantation, plasmapheresis or plasma exchange is used frequently to reduce the titer of anti-donor antibodies, although the titer threshold in stem cell transplantation is unknown, as is the effectiveness of this procedure.

One interesting observation in this study was that no patient with DSA developed acute GVHD, whereas 23.6% of patients without DSA had grade II-IV acute GVHD. The incidence of acute GVHD in double UCB transplantation is between 40-60%, whereas it is much lower in recipients of single UCB transplantation. One hypothesis linking our findings to the disparities in GVHD incidence is that patients with DSA more frequently had hematopoiesis derived solely from one of the two transplanted UCB units. This theory would also explain the delay in the time to neutrophil engraftment noted in the presence of DSA in our study, since single UCB transplantation is associated with longer time to engraftment when compared with double UCB transplantation. An alternative hypothesis is that some, but not all of the stem cell progenitors are eliminated by DSA, leading to delayed but dual engraftment of both transplanted UCB units. Even though our sample size was too small for statistical testing, it was interesting to note that among patients with DSA against only unit, those with the highest DSA titres had
engraftment by the other unit, while patients with the lowest titres had engraftment with affected unit, suggesting that the intensity of the DSA is relevant.

Why there was an excess of graft failure in patients with DSA against only one UCB unit also remains an unanswered question. If host-derived T cells are important, then perhaps spreading of immune responses against other mismatched HLA antigens or non-HLA antigens, under the influence of other HLA factors is possible.\textsuperscript{26} It is also possible that DSA were transferred with the other UCB unit,\textsuperscript{27} leading to a priming of donor-derived of co-transplanted T cells. Finally, it is feasible that the co-transplanted unit without DSA simply was insufficient alone to sustain long-term hematopoiesis.

Until recently, \textit{in vitro} crossmatching was used to determine compatibility between donors and recipients. The relationship between a positive crossmatch and graft rejection in allogeneic transplantation is well established.\textsuperscript{28} Gutman \textit{et al} have demonstrated that there is a relationship between the presence of pre-formed DSA and a positive crossmatch.\textsuperscript{7} With this in mind, they altered their UCB search strategy to avoid the use of these units, therefore were unable to correlate the presence of DSA and clinical graft failure. However, in contrast to their study, we used a much lower threshold for the detection of DSA (1000 MFI vs. 4000 MFI), and demonstrate that even low-titer antibodies are relevant in this setting for the prediction of graft failure. When examining an even lower threshold for the detection of DSA, Brunstein \textit{et al} were recently unable to detect a statistical difference in the rate of graft failure, despite a small difference in the rate of engraftment between those with DSA and those without (78% vs 86%, \( p = \text{NS} \)).\textsuperscript{29} This is consistent with our results that demonstrate that higher MFI was associated with an increased rate of failure to engraft. While our cutoff value of 1000 MFI can be used for future studies, further research in a larger sample size is required to determine the optimal cutoff value for cord blood selection.

Previous research in unrelated donor transplantation\textsuperscript{10} and in single unit UCB transplantation\textsuperscript{13,14} has demonstrated the correlation between the presence of DSA and graft rejection. This analysis demonstrates a correlation between the presence of DSA
and graft rejection, as well as other major transplantation outcomes, in double UCB transplantation. This has significant impact on search strategies for compatible UCB units for transplantation. Several factors must be considered when searching for the optimal unit, and we feel that the presence of DSA should be an important factor in the selection algorithm. Units against which there are very intense antibody responses should be particularly avoided. Given the adverse effect on survival, the use of two UCB units with preformed DSA in the recipient should be avoided as well.
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The Authors declare no relevant conflicts of interest.
References


Legend to Tables

Table 1. Baseline Patient Characteristics

NHL/CLL/HD = Non-Hodgkin Lymphoma / Chronic Lymphocytic Leukemia / Hodgkin Disease
AML = Acute Myelogenous Leukemia
MDS/MPD = Myelodysplastic Syndrome / Myeloproliferative Disease
ALL = Acute Lymphoblastic Leukemia
SAA = Severe Aplastic Anemia
CML = Chronic Myelogenous Leukemia
RIC Conditioning = Reduced Intensity Conditioning
Tac/Sir = Tacrolimus / Sirolimus

P values represent the comparison between No DSA vs. Any DSA.
N/A – not applicable

Table 2. Umbilical Cord Blood Product Characteristics

TNC = Total Nucleated Cells

Table 3 – Specificity and Intensity of DSA

MFI = Mean Fluorescence Intensity
Table 1. Baseline Patient Characteristics

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<td>48 (19 – 67)</td>
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<td>48 (19 – 63)</td>
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<td>AML</td>
<td>22 (30.1%)</td>
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<td>5 (6.9%)</td>
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<td>RIC Conditioning</td>
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<td>Tac/Sir GVHD Prophylaxis</td>
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Table 2. Umbilical Cord Blood Product Characteristics

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<th>DSA – Cord #2</th>
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<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt; cells infused (x 10&lt;sup&gt;5&lt;/sup&gt;/kg)</td>
<td>1.5</td>
<td>1.4</td>
<td>0.66</td>
<td>1.2</td>
<td>0.9</td>
<td>0.24</td>
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<tr>
<td>TNC infused (x 10&lt;sup&gt;7&lt;/sup&gt;/kg)</td>
<td>2.6</td>
<td>2.5</td>
<td>0.79</td>
<td>2.7</td>
<td>2.0</td>
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<td>Trypan Viability</td>
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<td>98%</td>
<td>0.58</td>
<td>95%</td>
<td>99%</td>
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<td>DSA</td>
<td>HLA Antigen</td>
<td>MFI</td>
<td>Engraftment Chimerism Outcome (Day 100)</td>
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<td>Cord Unit 1</td>
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<td>18 000</td>
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<tr>
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<td>B62</td>
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<td>Cw3</td>
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<tr>
<td></td>
<td>B51</td>
<td>5 500</td>
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<tr>
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<td>Cw7</td>
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<tr>
<td>Cord Unit 1 + 2</td>
<td>A23 / A1</td>
<td>2 200 / 1 900</td>
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<td></td>
<td>Cw7 / Cw7</td>
<td>2 300 / 2 300</td>
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<td></td>
<td>B35 / B37</td>
<td>23 000 / 12 500</td>
<td>Graft Failure</td>
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<td>B35 / B35</td>
<td>1 200 / 1 200</td>
<td>Dual chimerism</td>
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<tr>
<td></td>
<td>A2 / A29</td>
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<tr>
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<td>B38 / Cw7</td>
<td>2 700 / 12 300</td>
<td>Graft Failure</td>
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Legend to Figures

Figure 1. Cumulative Incidence of Graft Failure
Solid Line: No DSA
Dashed Line: DSA against one units
Dotted Line: DSA against both units

Figure 2. Cumulative Incidence of Neutrophil Engraftment
Solid Line: No DSA
Dashed Line: DSA against one or both units

Figure 3. Cumulative Incidence of Early Death or Relapse
Solid Line: No DSA
Dashed Line: DSA against one unit
Dotted Line: DSA against both units

Figure 4. Progression-Free (a) and Overall Survival (b)
Solid Line: No DSA
Dashed Line: DSA against both units
Figure 1. Cumulative Incidence of Graft Failure
Figure 2. Cumulative Incidence of Neutrophil Engraftment
Figure 3. Cumulative Incidence of Early Death or Relapse
Figure 4. Progression-Free (a) and Overall Survival (b)
Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation