Marked Increased Risk of Epstein-Barr Virus-Related Complications with the Addition of Anti-Thymocyte Globulin to a Non-Myeloablative Conditioning prior to Unrelated Umbilical Cord Blood Transplantation

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Abstract

Umbilical cord blood (UCB) is an increasingly utilized alternative source of hematopoietic stem cell for transplantation for patients who lack a suitable sibling donor. Despite concerns about a possible increased risk of Epstein-Barr Virus (EBV) lymphoproliferative disorder (PTLD) after UCB transplant, early reports documented rates of PTLD comparable to those reported after HLA matched unrelated marrow myeloablative (MA) transplants. To further investigate the incidence of EBV PTLD after UCB transplant and potential risk factors, we evaluated the incidence of EBV-related complications in 335 patients undergoing UCB transplant with a MA or non-myeloablative (NMA) preparative regimen. The incidence of EBV-related complications was a 4.5% overall, 3.3% for MA, and 7% for NMA transplants. However, the incidence of EBV-related complications was significantly higher in a subset of patients treated with a NMA preparative regimen that included anti-thymocyte globulin (ATG) versus those that did not (21% vs. 2%, p<0.01). Nine of 11 patients who developed EBV PTLD were treated with rituximab (anti-CD20 antibody), with the five responders being alive and disease free at a median of 26 months. Use of ATG in recipients of a NMA preparative regimen warrants close monitoring for evidence of EBV reactivation and potentially preemptive therapy with rituximab.

Word Count: 199
Introduction

Umbilical cord blood (UCB) has become a valuable alternative for patients who require hematopoietic stem cell transplantation (HSCT) but who lack an HLA-matched sibling donor. Compared with unrelated adult donors, UCB is readily available, has a low risk of infection transmission, and has lower than expected incidence of graft-vs.-host disease (GVHD) considering the degree of HLA mismatch.

Epstein Barr Virus (EBV)-viremia and post-transplant lymphoproliferative disorder (PTLD) are well recognized complications of allogeneic transplantation HSCT. These complications have been associated with unrelated donor transplants, HLA mismatch, anti-thymocyte globulin (ATG) administration, and ex-vivo or in vivo T-cell depletion. Despite concerns regarding immune reconstitution, and case reports of EBV PTLD following UCB transplantation (UCBT), a retrospective analysis at two institutions found the incidence of EBV PTLD after a myeloablative (MA) preparative therapy and UCB transplant to be low. A recent analysis found no significant difference in the risk of serious viral infections, including PTLD, in recipients of unrelated donor UCB or unmanipulated marrow. However, an increased number of cases of EBV PTLD has been observed recently at our center leading to a new analysis of EBV-related complications in our patient population transplanted with UCB with the aim of assessing incidence and identifying potential risk factors.

Methods

Patients and UCB Grafts

Three-hundred-thirty-five consecutive patients who underwent UCB transplantation (UCBT) at the University of Minnesota Medical Center-Fairview and University of Minnesota Children’s Hospital-Fairview between July 1994 and March 2005 were
included in this analysis. Median age was 16 years (0.2-69); median weight 53.7kg (3.8-134.0); and median follow-up was 1.2 years (range: 77 days–9.2 years). Patients were stratified according to type of preparative therapy. Compared to recipients of non-myeloablative (NMA) regimen, patients treated with a MA preparative regimen were significantly younger (median 8 yrs vs. 50 yrs, p<0.01), had lower weight (median 30kg vs. 78kg, p=0<.01), and had longer median follow-up (1.5 yrs vs. 1.2 yrs, p=0.02). Grafts were matched at 4 to 6 out of 6 at HLA loci (HLA A, B [intermediate resolution] and DRB1 [high resolution]) to the recipient, except one with 3 of 6 HLA matched graft. One hundred twenty-six (38%) patients received two UCB units; 240 (72%) received a MA preparative regimen; 250 were transplanted for a malignant disease. Of the 85 patients who were transplanted for a non-malignant disease, 83 received a MA preparative regimen. The median infused total nucleated cell dose (TNC) was significantly higher among recipients of a MA preparative regimen (4.1 vs. 3.6 X 10^7/kg, p<0.01). Median CD34 cell dose was 4.4 X10^5 cells/kg (range: 0.4-96.7), and was similar for recipients of MA and NMA preparative regimens. All transplant protocols were approved by the University of Minnesota Institutional Review Board. All patients or their legal guardians provided written informed consent for the transplantation procedure.

**Preparative Regimen**

The MA preparative regimen included cyclophosphamide regimen with either busulfan or total body irradiation (TBI) and equine ATG (ATGAM, Pharmacia, Kalamazoo, MI) in 174 (73%). ATG was administered at 15 mg/kg every 12 hours for 6 doses on days -5 through -3. The NMA preparative regimen consisted of cyclophosphamide, fludarabine and TBI 200 cGy as detailed elsewhere. After April 2002, 30 patients (32%) who had not received multi-agent chemotherapy in the preceding 3 months (excluding those with prior autologous transplant), received ATG as additional pre-transplant immune suppression. ATG was incorporated into the NMA therapy for these patients without
recent chemotherapy because of a higher incidence of graft failure. ATG was initially administered at 15 mg/kg every 12 hours for 6 doses on days -3 through -1, and moved to days -6 through -4 in November 2004.

**Post-transplant Immunosuppression and GVHD Therapy**

All patients received post-transplant immunosuppression with either cyclosporine A (CsA)/mycophenolate mofetil (MMF) (50%), CsA/methotrexate (1%), or CsA/methylprednisolone (49%). CsA and MMF were administered in the same dose and schedule for the MA and NMA settings. CsA was administered twice daily with a target trough level 200-400 ng/mL, measured by HPLC whole blood mass spectroscopy. MMF was administered at 1 g twice daily between days -3 and +30, with no taper.

Methotrexate was administered in the MA at 15 mg/m² on day 1, and 10 mg/m² on days 3, 6, 11. Methylprednisolone was administered in the MA at 1 mg/kg, every 12 hours, between days +5 and +19, with subsequent taper. Grades II to IV acute GVHD was treated with CsA, target levels as above, and prednisone 60 mg/m²/day for 7 days, followed by a rapid eight week taper. Extensive chronic GVHD was treated with CsA (target level as above), methylprednisolone 15 mg/kg as a bolus intravenous injection weekly for 8 weeks, and prednisone 0.5 mg/kg on alternate days for 12 months, followed by a slow taper. During GVHD therapy patient received antimicrobial, antiviral, antifungal, and Pneumocystis prophylaxis as described below.

**Antiviral Prophylaxis/Supportive Care after UCBT**

Patients who were cytomegalovirus IgG antibody seropositive prior to transplantation received antiviral prophylaxis with high dose acyclovir [800 mg (18 mg/kg for children) orally 5 times daily or 10 mg/kg intravenously 3 times daily]. Antifungal and antibacterial prophylaxis was provided using fluconazole and penicillin or levofloxacin, respectively.
Selected patients at high risk for the development of filamentous fungal infection, such as those with an underlying condition of myelodysplastic syndrome, aplastic anemia, heavily pretreated acute leukemia, or those with fungal infection prior to transplantation, received antifungal prophylaxis with voriconazole in place of fluconazole. All patients received G-CSF from the day of transplant until 2500 neutrophils/µL. Irradiated filtered blood products and parenteral nutrition were administered according to institutional guidelines. Pneumocystis prophylaxis was initiated following engraftment.

**EBV Assay**

Through 2003, blood for quantitative EBV polymerase chain reaction (PCR) testing was performed off-site, using primers for the EBNA-1 gene (Eastern Virginia Medical School, Norfolk, VA). The EBV PCR assay included positive and negative control samples. The lower limit of detection of the assay was 100 copies of viral DNA per 100,000 cells. At the beginning of 2004, most testing was performed on site at the University of Minnesota, using real-time TaqMan PCR. The amplicon was a 71-bp portion of the EBNA-1 gene. Quantitative EBV data were expressed as viral copies per milliliter. The limit of detection of the assay was 10 viral copies/reaction.

**EBV Related Disease and Rituximab Therapy**

EBV viremia was defined as >1,000 copies of EBV DNA per mL of whole blood. EBV PTLD was defined as biopsy or autopsy proven post-transplant lymphoma, or viremia along with computerized tomography nodal or soft tissue abnormalities consistent with PTLD. Patients treated with rituximab received 375 mg/m², weekly for four weeks.
Statistical Considerations

The cumulative incidence of EBV-related complications was estimated by treating deaths from other causes as competing risks. Survival after documented EBV-viremia or PTLD was estimated by the Kaplan-Meier method. Comparison of incidence between subgroups used the Log-Rank test. Cox regression analysis was performed to test the independent effect of factors on EBV-related complications. Events were analyzed as of October, 2005. Statistical comparison of continuous factors was performed by the Wilcoxon Two-Sample test or the Kruskal-Wallis test. Differences in categorical factors were tested across subgroups by the use of the Chi-square test or Fisher’s exact test.

Results

EBV-Related Events

Fifteen of 335 patients developed EBV-related complications at a median of 133 days (52-407) after UCB transplantation. A summary of the 15 cases of EBV-related complications is detailed in Table 1. Four had viremia and 11 PTLD involving bone marrow, lymph nodes, tonsil, liver, skin, stomach, and lung. Among 11 patients who developed EBV PTLD, 5 survive 113-1668 days after UCB transplant. Five of 9 treated with rituximab responded to therapy and survive. At 1 year, 45% (95% CI, 15-75%) survive following the diagnosis of EBV-related complication. The overall incidence of EBV viremia and EBV-associated PTLD was 4.5%, similar to that previously reported. The incidence of EBV-viremia and EBV-associated PTLD was 3.3% and 7.4% in recipients of MA and NMA preparative therapy, respectively. Demographic characteristics of the two groups are summarized in Table 2. In the univariate analysis, age, sex, CMV serostatus, number of UCB units composing the graft, prior autologous transplant, and disease group (malignant or non-malignant) were not significantly associated with the incidence of EBV-related complications. An increased risk, however,
was observed with HLA-mismatch (p=0.03). In contrast to our prior report where all patients had received ATG as part of a MA conditioning, a higher incidence of EBV-associated complications was found in patients treated with ATG (14/204 [7%]) as compared to those without ATG (1/131 [0.8%], p=0.02). As shown in Table 3, patients who received ATG as part of the preparative regimen had a significantly lower incidence of acute GVHD, but the incidence of EBV-related complications was similar between patients who did and did not develop grades II-IV acute GVHD ( 7/149 [4.6%] vs. 8/186 [4.3%], p=0.86). Table 3 and summarizes outcomes by preparative regimen and administration of ATG as part of the preparative regimen.

EBV-Related Events after a MA Therapy

Among 240 patients who received a MA preparative regimen, the incidence of EBV viremia or PTLD was 3.3% (95% CI, 1.0-5.6%). However, all eight cases were observed among the 174 patients who received ATG as part of the preparative regimen, and in none of 166 who did not. This difference, however, was not statistically significant (p=0.08) (Figure 1A). As it is shown in Table 2, patients who received a MA preparative regimen with ATG were more likely to be younger, weigh less, CMV seronegative, and recipient of a single unit UCB unit. The median time to the development of EBV-related complications was 102 days (range: 52-407). Patients who received ATG were less likely to develop grades II-IV acute GVHD and extensive chronic GVHD (Table 3). There was no significant difference on the proportion with primary neutrophil engraftment and survival between patient who did or did not receive ATG as part of the preparative regimen (Table 3 and Figure 2A).
EBV-Related Events after a NMA Therapy

Among 95 patients who received a NMA preparative regimen, the incidence of EBV viremia or PTLD was 7% (95% CI, 2-14%). However, there was a significantly a higher risk among patients who received ATG (21% vs. 2%, p<0.01) (Figure 1B). Among 30 patients who received ATG, five developed EBV PTLD and 1 EBV viremia. Patients who received a NMA preparative regimen with ATG were more likely to be older, male, weigh more and be a recipient of two UCB units (Table 2). Among patients who received a NMA preparative regimen, the median time to the development of EBV-related complications was 133 days (range: 54-603) with EBV PTLD occurring at a median of 133 days (range: 54-247). Patients who received ATG were less likely to develop grades II-IV acute GVHD (Table 3). There was no significant difference on the proportion with primary neutrophil engraftment, extensive chronic GVHD and survival between patient who did or did not receive ATG as part of the preparative regimen (Table 3 and Figure 2B).

As shown in Table 4, Cox multivariate regression analysis the only independent predictor of an increased risk of EBV-related complications was a NMA preparative regimen with ATG (RR 15.4, 95% CI, 2-116, p<0.01). Neither prior CMV serostatus, HLA match, nor GVHD prophylaxis were predictors of EBV-related complications.

Discussion

An unexpectedly high incidence of EBV-related complications has recently been observed for patients undergoing UCB transplant with a NMA preparative regimen including ATG. As increasing transplant centers use a NMA preparative regimen in the context of UCB transplantation, there needs to be an awareness of this new risk. At our center, the magnitude of the risk necessitated patient notification in those previously...
treated and alteration of the consent for those receiving ATG. Further, these observations demand closer monitoring in recipients UCB and ATG for evidence of EBV reactivation after transplant and the consideration of pre-emptive anti-CD20 therapy.

In this study, we found a cumulative incidence of EBV-related complications of 4.5% and EBV PTLD of 3%. This rate is similar to the 2% incidence of EBV PTLD in the combined datasets of the University of Minnesota and Duke University Medical Center previously reported. Furthermore, this result compares favorably with the incidence of EBV PTLD observed after other allogeneic stem cell sources which ranges from < 1% to 29% depending upon the use of T cell depletion, post transplant immune suppression and HLA match between the donor and recipient. Higher risks have been associated with unrelated donor, T-cell depletion (TCD), HLA-mismatch, and administration of ATG. A recent report showed a significantly higher incidence of EBV-related complications after a NMA preparative regimen. In contrast to our series, this study included only children, 41 of 65 patients had primary immunodeficiency, and grafts were from matched and mismatched related and unrelated donors.

Until the mid 1990’s, quantitative measurements of EBV load were not determined routinely. This may account for the lower incidence of viremia reported in earlier series. More recently, patients with persistent fever with or without associated adenopathy had EBV viral load measurements. Six of 15 patients who developed EBV-related complications, were diagnosed by the EBV assay using real-time TaqMan PCR. Although this is a more sensitive technique, it is not likely to explain our findings as in our retrospective analysis as EBV PCR was only obtained for those patients with clinical manifestations suspicious for EBV reactivation, and patients were not being routinely monitored for EBV reactivation. This is also evidenced by the fact that two patients were
found to have EBV-PTLD on autopsy. After allogeneic HSCT, EBV viremia is a frequent event, with incidence ranging between 29% and 65% \(^4,6,8-11\), and increased risk has been associated with unrelated donor, TCD and administration of ATG \(^4,6,8,10,11,23\). The effect of donor source on EBV viremia has not been reported.

Importantly, all but one case of EBV-related complications were diagnosed in patients who received ATG as part of their preparative regimen, particularly after a NMA preparative regimen. The 21% incidence found in the subgroup who received ATG with NMA conditioning far exceeds the expected overall low risk after UCB transplant \(^22\), similar to what has recently been reported for children receiving adult derived HSC with ATG or Campath as part of a NMA preparative regimen \(^23\). Clave et al have shown that patients who have EBV-specific T cells at the onset of reactivation are more likely to control the viral reactivation without additional therapy \(^11\). The absence of EBV-specific memory T-cells in UCB grafts, more frequent use of HLA-mismatch grafts, and incorporation of ATG inducing *in vivo* TCD may all contribute to a higher risk of EBV-related complications in this subset of patients after UCBT \(^11\). However, as we observed no increased risk of EBV complications after a MA preparative regimen, even with ATG, other factors such as patient age, diagnosis, prior therapy, and nucleated cell dose may modify the risk. Furthermore, it is possible that following NMA conditioning the number of residual recipient B-cells may play a role. In multivariate analysis, the only independent predictor of an increased risk of EBV-related complications was a NMA preparative regimen with ATG. In our cohort, HLA-mismatch itself, a risk factor for EBV PTLD for other HSC sources \(^13,16,20\), does not appear to be a predictor for EBV-related complications.
Rituximab is an anti-CD20 humanized monoclonal antibody which has been shown to active against malignant $^{37-39}$ and non-malignant $^{40-45}$ B-cell diseases. Recent reports have shown its activity against EBV PTLD $^{8,9,11,23}$. Rituximab was administered in nine of 15 patients who developed EBV-related complications, with 5 responding and all 5 alive and EBV disease free beyond 1 year. One of the rituximab responders had also received methylprednisolone. Among the 4 patients who failed rituximab, 3 received the drug alone and one in combination with vincristine.

Reduction of immune suppression, administration of anti-CD20 antibody and donor lymphocyte infusions (DLI) have been used for the treatment of EBV PTLD with variable success $^{5,7,9,46-48}$. One of the limitations of UCB transplantation, is the unavailability of donor lymphocytes for treatment of EBV PTLD or relapse. Alternatives for recipients of UCB would be 1) elimination of ATG from the preparative regimen, 2) addition of rituximab to eliminate B cells, or 3) incorporation of an agent that eliminates both B and T cells, such as alemtuzumab (Campath-1H). Campath-1H has been associated with a lower risk of EBV complications than ATG $^{13,23}$. However, Campath-1H has been associated with opportunistic infections, particularly, viral reactivation including CMV $^{49-52}$, and loss of complete chimerism $^{53-55}$. However, in recipients of UCB, early diagnosis, reduction of immune suppression when possible and use of rituximab are the principal options in those with EBV-related complications.

Alternatively, monitoring for EBV with therapeutic intervention only in those with increasing viral load may be a safer approach. More recently, studies in HSCT $^{7-11,23,56}$ as well as solid organ transplantation $^{56-60}$ suggest that EBV viral load monitoring may be worthwhile in high risk populations. Some suggest that preemptive therapy is highly effective in controlling viral proliferation and avoiding progression into EBV PTLD $^{7-10,57}$. 
After HSCT, rituximab seems to be effective preemptive therapy once viremia is detected, but of lesser efficacy once EBV PTLD is fully established. It is suggested that patients with reduction in viral load after a single dose of rituximab are likely to become complete responders, while a rising load is predictive of failure. Close viral monitoring during therapy may be valuable, particularly in high risk populations.

Patients undergoing a NMA UCBT with ATG are at uniquely higher risk for the development of EBV-related complications, in particular PTLD. Although reduction of immune suppression should always be considered, donor lymphocyte infusions are not available from the UCB donor. Recent data suggest that EBV viral load monitoring with pre-emptive rituximab treatment for those who develop viremia may halt the progression to PTLD. Therefore, patients who receive ATG as part of a NMA preparative regimen should have quantitative EBV monitoring between days 30 and 180 after UCBT. At our institution, patients who develop EBV-viremia (>1,000 copies of EBV DNA per mL of whole blood) receive therapy with a single dose of rituximab. Patients with persistent viremia or evidence of PTLD receive more aggressive therapy with additional doses of rituximab with or without chemotherapy. Regardless, all potential recipients of UCB and ATG must appropriately counseled on this potential risk.
References

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Figure 1. Cumulative incidence of Epstein-Barr Virus-related complications (A) after myeloablative (n=240) and (B) after non-myeloablative (n=95) umbilical cord blood transplantation.

A. Myeloablative

B. Non-myeloablative
Figure 2. Kaplan-Meier probability of overall survival (A) after myeloablative (n=240) and (B) after non-myeloablative (n=95) umbilical cord blood transplantation.

A. Myeloablative

B. Non-myeloablative
Table 1. Characteristics of patients who developed EBV-related complications.

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* Liver, marrow, and skin biopsies.
** Liver, tonsil, and lung biopsies.

ALD, adrenoleukodystrophy; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CT scan, computerized tomography; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; CMV, cytomegalovirus; CsA, cyclosporine A; GVHD, graft-vs.-host disease; MA, myeloablative; MDS, myelodysplastic syndrome; M-pred, Methylprednisolone; MLS, Maroteaux-Lamy syndrome; MOF, multiple organ failure, NMA, non-myeloablative; OP, osteopetrosis; PTLD, post-transplant lymphoproliferative disorder; PCR, polymerase chain reaction; SAA, severe aplastic anemia.
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<th>Myeloablative (n = 240)</th>
<th>Non-Myeloablative (n = 95)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>8 (0.2-53)</td>
<td>50 (18-69)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Weight in kg</td>
<td>30 (4-120)</td>
<td>78 (50-134)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Recipient CMV Positive</td>
<td>108 (45%)</td>
<td>47 (49%)</td>
<td>0.46</td>
</tr>
<tr>
<td>HLA match</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6</td>
<td>28 (12%)</td>
<td>6 (6%)</td>
<td>0.02</td>
</tr>
<tr>
<td>5/6</td>
<td>109 (45%)</td>
<td>29 (31%)</td>
<td></td>
</tr>
<tr>
<td>3-4/6</td>
<td>103 (43%)</td>
<td>60 (63%)</td>
<td></td>
</tr>
<tr>
<td>ATG</td>
<td>Yes</td>
<td>174 (73%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number of UCB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Unit</td>
<td>192 (80%)</td>
<td>17 (18%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Double Unit</td>
<td>48 (20%)</td>
<td>78 (82%)</td>
<td></td>
</tr>
<tr>
<td>Median Infused Nucleated Cell Dose X 10^7/kg (range)</td>
<td>4.1 (0.7-28.1)</td>
<td>3.6 (1.1-6.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Median Infused CD34+ Cell Dose X 10^7/kg (range)</td>
<td>4.3 (0.4-96.7)</td>
<td>4.5 (0.7-18.8)</td>
<td>0.57</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>157 (65%)</td>
<td>93 (98%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Years of Follow-up</td>
<td>1.5 (0.3-9.2)</td>
<td>1.2 (0.3-3.5)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ATG, anti-thymocyte globulin; CMV, cytomegalovirus; HLA, human major histocompatibility complex; UCB, umbilical cord blood.
Table 3. Outcomes of the 335 umbilical cord blood transplant patients by preparative and administration of ATG

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Myeloablative no ATG (n = 66)</th>
<th>Myeloablative with ATG (n = 174)</th>
<th>p value</th>
<th>Non-Myeloablative No ATG (n = 65)</th>
<th>Non-Myeloablative ATG (n = 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(95% confidence interval)</td>
<td>(95% confidence interval)</td>
<td></td>
<td>(95% confidence interval)</td>
<td>(95% confidence interval)</td>
<td></td>
</tr>
<tr>
<td>Grade II-IV</td>
<td>58% (45-71%)</td>
<td>34% (27-41%)</td>
<td>&lt;0.01</td>
<td>63% (49-77%)</td>
<td>37% (19-55%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>Extensive</td>
<td>20% (10-30%)</td>
<td>&lt;0.01</td>
<td>28% (16-40%)</td>
<td>21% (5-37%)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Chronic GVHD (1 yr)</td>
<td>97% (93-100%)</td>
<td>92% (88-96%)</td>
<td>0.17*</td>
<td>92% (86-98%)</td>
<td>94% (84-100%)</td>
</tr>
<tr>
<td></td>
<td>Primary Neutrophil Engraftment</td>
<td>60% (56-76%)</td>
<td>52% (44-60%)</td>
<td>0.58</td>
<td>46% (32-60%)</td>
<td>45% (25-65%)</td>
</tr>
</tbody>
</table>

* Comparison of proportions at day 42 post-umbilical cord blood transplant.
ATG, anti-thymocyte globulin; GVHD, Graft vs. host disease.
<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative Risk (95% C.I.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conditioning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloablative*</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Non-myeloablative (no ATG)</td>
<td>0.7 (0.1-6.5)</td>
<td>0.51</td>
</tr>
<tr>
<td>Non-myeloablative (ATG)</td>
<td>15.4 (2.0-116.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>CMV Serostatus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive*</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>3.0 (0.9-9.7)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>HLA (engrafted in doubles)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5/6</td>
<td>0.2 (0.1-1.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>3&amp;4/6</td>
<td>0.9 (0.2-4.7)</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>Number of Donors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.4 (0.1-2.4)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Factors included in the model and tested for proportional hazards were conditioning regimen, cytomegalovirus serostatus, age, weight, Graft vs. Host disease prophylaxis, cell dose, diagnosis, number of UCB donor units, HLA match, prior autologous transplant, and sex.

ATG, anti-thymocyte globulin; EBV, Epstein-Barr Virus; CMV, cytomegalovirus
Marked increased risk of epstein-barr virus-related complications with the addition of anti-thymocyte globulin to a non-myeloablative conditioning prior to unrelated umbilical cord blood transplantation

Claudio G Brunstein, Daniel J Weisdorf, Todd DeFor, Juliet N Barker, Jakub Tolar, Jo-Anne H van Burik and John E Wagner