Determinants of Engraftment after Double-Unit Cord Blood Transplantation

Doris M. Ponce, MD, and Juliet N. Barker, MBBS, FRACP

CORD BLOOD (CB) IS AN ALTERNATIVE SOURCE OF ALLOGENEIC STEM CELLS FOR HEMATOPOIETIC TRANSPLANTATION, ESPECIALLY FOR PATIENTS WHO LACK A SUITABLE HLA-MATCHED RELATED OR UNRELATED VOLUNTEER DONOR OR ARE IN URGENT NEED OF A TRANSPLANT. However, one of its major limitations is the low total nucleated cell (TNC) dose per kilogram recipient body weight, especially in adults and larger children. In addition, the limited size of the global inventory frequently forces clinicians to transplant units with a high degree of HLA mismatch, which further compounds the problem of low cell dose. Numerous studies have demonstrated an increased risk of delayed engraftment, graft failure, and transplant-related mortality (TRM) in CB transplantation (CBT) recipients who receive a low cell dose. More recently, the adverse impact of HLA mismatches has been fully appreciated. In particular, the recognition of the interaction between TNC dose and HLA mismatch dictates that for an increasing degree of HLA-A and -B antigen and -DRB1 allele mismatch, a higher TNC dose is required to achieve a similar overall survival. For example, the study by Barker et al revealed a cryopreserved TNC dose of $\geq 5.0 \times 10^7$/kg was...
required in single-unit CBT recipients with two HLA mismatches to achieve a TRM and survival similar to CBT recipients with one HLA mismatch and a TNC dose \( \leq 2.5 \times 10^7/\text{kg} \). Such cell doses cannot be achieved in the majority of larger children and nearly all adults, and it will take extensive funding and a considerable period of time to sufficiently increase the global public CB inventory to enable the provision of more-closely matched units to the majority of patients. For these reasons, numerous strategies to augment graft cell dose are being investigated. One such strategy, and the focus of this chapter, is the use of double-unit grafts.

### Early Experience with Double-Unit Transplantation

Double-unit CBT was originally developed as a platform to investigate the manipulation of one of the two units, with the unmanipulated unit acting as an “in-vivo backup” unit to guard against the risk of graft failure. However, given it was unknown whether there would be crossed immunologic rejection, double-unit CBT with both units unmanipulated was the first step in the investigation of this strategy. The first double-unit CBT was performed by the group at the University of Minnesota in 2000, and successful sustained donor-derived engraftment was achieved, demonstrating the feasibility and safety of this method. Barker et al then reported two series of double-unit CBT in recipients of myeloablative and nonmyeloablative conditioning for the treatment of high-risk or advanced hematologic malignancies. After myeloablation, the median time to neutrophil engraftment was 23 days (range = 15 to 41 days), with all evaluable patients achieving sustained donor-derived neutrophil engraftment. The striking finding was that the majority of patients engrafted with only one of the two units, as assessed by analysis of the bone marrow 21 days after transplantation. Despite this, the incidence of neutrophil engraftment after double-unit CBT was better than historical controls, raising the possibility that the nonengrafting unit could somehow augment the engraftment of the dominant unit. Furthermore, 1-year overall survival in this series was 72%, introducing double-unit CBT as a viable alternative to unrelated-donor graft transplantation. These results have been replicated at other centers, such as Memorial Sloan-Kettering Cancer Center (MSKCC). For example, in 54 double-unit CBT recipients with high-risk or advanced hematologic malignancies (median age = 42 years; range = 7-66), the incidence of sustained donor engraftment was 94%, and the 1-year overall survival was 65% with a disease-free survival of 56% (Fig 26-1).

Improved engraftment after double-unit CBT compared with single-unit CBT is yet to be proven. The early series of double-unit CBT reported by the University of Minnesota used novel fludarabine-based conditioning, omitted antithymocyte globulin, and switched the immunosuppression from cyclosporin A plus corticosteroids to cyclosporin A with mycophenolate mofetil. These changes could have enhanced engraftment independent of the graft. Thus, a randomized study of single- vs double-unit CBT using this novel conditioning and immunosuppression regimen is currently being conducted by the United States Clinical Trials Network to test the specific contribution of the double-unit graft in children. However, this study requires a single unit of at least \( \geq 2.5 \times 10^7/\text{kg} \) to permit enrollment and randomization to the transplantation of a second unit as a double-unit graft. Thus, it will not answer questions about the utility of double-unit CBT in adults. Moreover, randomization of adult CBT recipients to a single- vs double-unit trial is not appealing because it is now routine to expect a sustained engraftment rate in double-unit CBT recipients of approximately 93% to 95% in patients with hematologic malignancies. This engraftment rate is remarkable given the relatively low TNC dose and degree of HLA mismatch of the unit responsible for
Figure 26-1. One-year overall survival and disease-free survival in double-unit cord blood transplantation recipients (n = 54).
sustained donor engraftment. What may be of even greater interest, however, is the suggestion from multiple series that double-unit CBT may be associated with a decreased incidence of malignant relapse. If confirmed in controlled prospective studies, this would introduce double-unit CBT as a strategy to improve CBT survival independent of engraftment and makes the study of the biology of double-unit engraftment even more compelling. When analyzing double-unit engraftment biology, two related but separate issues must be considered: 1) the mechanism of unit predominance and 2) the determinants of the speed and success of sustained donor-mediated engraftment.

Determinants of Unit Dominance

The determinants of unit dominance after double-unit CBT remain to be fully elucidated. Infused TNC and CD34+ cell doses, gender match, ABO blood group, HLA match, and order of infusion do not predict which unit will be dominant. Early clinical series reported that although there was no association between unit dominance and infused TNC or CD34+ cell doses, engrafting units had a higher infused CD3+ cell dose, supporting a role for T cells in unit dominance. More recently, Scaradavou et al analyzed unit quality in 46 double-unit CBT recipients, as measured by the percentage of viable CD34+ cells after thawing. They found that engrafting units almost always had CD34+ cell viability >75%, units with low viability were very unlikely to engraft (p = 0.0006), and poor CD34+ cell viability correlated with lower colony-forming unit (CFU) content (p = 0.02). (See Tables 26-1 and 26-2.) Interestingly, in this study the only patient that had graft failure received a graft in which both units had poor CD34+ cell viability. This suggests that one of the mechanisms by which double-unit CBT is effective is an increased likelihood of infusing at least one unit with good viability and, therefore, engraftment potential. The reason the importance of CD34+ cell viability had not been previously appreciated was likely that the use of a modified gating strategy in the Scaradavou series excluded only debris but gated in all dead cells. Using this approach, the percentage of live cells after thawing of the total number originally present in the graft can be calculated. Hence a distinction can be made between the two units shown in Fig 26-2. In this example, the two units have similar CD34+ cell doses/kg but have a significant difference: unit 1 has a high percentage of dead cells and thus a low CD34+ cell viabil-

<table>
<thead>
<tr>
<th>CD34+ Cell Viability</th>
<th>Dominant Unit (n = 44)</th>
<th>Nonengrafting Unit (n = 44)</th>
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<tbody>
<tr>
<td>&lt;75% (n = 16)</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>≥75% (n = 72)</td>
<td>43</td>
<td>29</td>
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Units with viability <75% were very unlikely to engraft.
7-AAD = 7-amino-actinomycin D.
Table 26-2. Factors Associated with Engraftment in Analyses* of Double-Unit Cord Blood Transplantation\textsuperscript{10,16}

<table>
<thead>
<tr>
<th>Engraftment Parameter</th>
<th>Finding</th>
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<tr>
<td>Unit dominance</td>
<td>Units with CD34(^+) cell viability $&lt;75%$ unlikely to engraft</td>
</tr>
<tr>
<td></td>
<td>Dominant units have higher CD3(^+) cell doses</td>
</tr>
<tr>
<td>Speed and success of engraftment</td>
<td>Low CD34(^+) cell viability is associated with increased graft failure risk</td>
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<td></td>
<td>Higher CD34(^+) cell and CFU doses in the dominant unit predict enhanced engraftment speed and success (but do not predict which unit will engraft)</td>
</tr>
<tr>
<td></td>
<td>Total graft TNC and CD3(^+) cell dose are also associated with engraftment speed and success</td>
</tr>
<tr>
<td></td>
<td>Unit/recipient HLA match has no influence on unit dominance or engraftment success</td>
</tr>
<tr>
<td></td>
<td>Unit/unit HLA match has no influence on engraftment success but influences how long ultimately nonengrafting units can be detected after transplantation</td>
</tr>
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*From the Memorial Sloan-Kettering Cancer Center. 
CFU = colony-forming unit; TNC = total nucleated cell (dose).

Figure 26-2. Comparison of two units with similar CD34\(^+\) cell doses but highly disparate percentages of viable CD34\(^+\) cells.\textsuperscript{16} Unit 2 has a high percentage of viable CD34\(^+\) cells after thawing and therefore is of better quality. It would likely dominate in engraftment if these units were transplanted as a double-unit graft.
ity, and unit 2 has a high percentage of viable CD34+ cells.

Georges et al have confirmed that higher CD34+ cell viability was the only graft variable to predict unit dominance in a canine model of double-unit CBT.17 The importance of CD34+ cell viability has been further substantiated in an updated MSKCC series of 84 double-unit CBT recipients transplanted for hematologic malignancies.10 In this study, 79 patients had sustained donor engraftment, whereas five patients had hematopoiesis identified from a dominant unit, but this did not result in clinical engraftment. The significant findings of this study are summarized in Table 26-2. Notably, when the CD34+ cell viability of the dominant unit in the 79 engrafters was compared with that of the five patients with clinical graft failure, a CD34+ cell viability ≥75% in the dominant unit was associated with an increased likelihood of sustained donor engraftment (p = 0.03). Although these analyses demonstrate that units of low CD34+ cell viability are unlikely to engraft, the mechanism of unit dominance when two units of adequate viability are coinfused remains to be fully elucidated.

Murine studies have been performed to investigate double-unit CBT biology. Kim et al evaluated the effect of transplanting mononuclear cells (MNCs) from two CB units into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice.18 Despite each unit engrafting alone, coinfusion of MNCs as a double-unit graft was associated with predominance of one CB unit. Unit dominance could be mitigated (and coengraftment achieved) with either lineage depletion of the units or cotransplantation of third-party marrow-derived mesenchymal stromal cells, suggesting that immune-mediated graft-vs-graft interactions play a critical role in unit dominance. Using NOD/SCID/interleukin-2 receptor (IL-2R)γnull mice, Yahata et al replicated the observation that the coinfusion of MNCs from two CB units resulted in single unit dominance, whereas mixed chimerism could be generated by CD34+ selection.19 Additional experiments by this group extended this observation by demonstrating that unit dominance was T-cell mediated and specifically involved the cooperation of CD4+ and CD8+ cells.

Eldjerou et al were the first to report both in-vitro and in-vivo studies of double-unit CBT using aliquots of cells from each unit of a clinical double-unit graft and correlation of the laboratory findings with patient engraftment.20 They found that the hematopoietic potential of each unit in vitro, as measured by colony-forming cell and cobblestone-area-forming cell content using either MNCs or CD34+ cells, did not correlate with clinical unit dominance, and the contribution of each unit in cocultures was concordant with the clonogenic efficiency of that unit when cultured alone. The experimental design of their NOD/SCID/IL-2Rγnull murine studies is shown in Fig 26-3, and results are summarized in Table 26-3. They found that although MNCs from either unit engrafted alone, when transplanted as a double-unit graft, not only was there single-unit dominance, but this correlated with clinical engraftment in 86% of the cases (p <0.001). Double-unit transplantation of CD34+ cells was associated with loss of both unit dominance and clinical correlation, whereas addition of CD34+ cells from only one of the two units restored unit dominance but with engraftment being mediated by the origin of the CD34+ cells, regardless of which unit engrafted in the patient. These findings suggest that unit dominance is an in-vivo event that is mediated by graft-vs-graft interactions, and given that clinical correlation can be observed in a murine model, it is not related to interactions between the graft and the host or a random event.

Clinical data has recently emerged that further supports unit dominance being immune mediated. In the Avery series, the association between a higher infused CD3+ dose/kg and unit dominance was again confirmed (Table 26-2).10 However, of greater interest were the observations in relation to HLA matching. As shown in Fig 26-4, investi-
Figure 26-3. Design of murine experiments investigating double-unit cord blood transplantation using research aliquots from clinical grafts. BM = bone marrow; DCB = double-unit cord blood; hu = human; MNC = mononuclear cell; SP = spleen.
gators evaluated high-resolution HLA-matching (10 alleles) of each unit of 79 recipients of double-unit CBT with sustained donor engraftment, and it was found that the dominant unit was not necessarily a better HLA match to the recipient when compared to the nondominant unit of the double-unit pair. However, the unit/unit HLA match influenced the length of time the ultimately nonengrafting unit could be detected (Tables 26-2 and 26-4). Specifically, recipients of double-unit grafts in whom the unit/unit HLA match was ≥7/10 HLA alleles matched were significantly more likely to have initial coengraftment and transient persistence of the ultimately nonengrafting unit, with one patient having sustained engraftment of both units long term. By contrast, recipients of units highly mismatched (≤6/10) to each other were more likely to have engraftment with only a single unit. This is likely caused by an enhanced unit-vs-unit immune response, whereas closely HLA-matched units are more likely to be relatively tolerant of each other, and in this setting, at least transient coengraftment is possible.

Additional evidence in favor of an immune basis for unit dominance comes from the study by Gutman et al, who demonstrated the presence of CD8+ T cells derived from the dominant unit that recognized the nondominant unit in the peripheral blood at 28 days after transplantation in nine of 10 double-unit CBT recipients with single unit dominance, regardless of the conditioning regimen.21 Interestingly, the three patients that had persistent mixed chimerism did not develop an alloreactive CD8+ T-cell response. Furthermore, it can be hypothesized that the failure of sustained engraftment of ex-vivo-expanded CB units in clinical trials of double-unit CBT in which one of two units is expanded22 did not result from failure to expand or maintain sufficient progenitors in the manipulated unit but from the expanded unit being T-cell depleted and therefore unable to compete against the T-cell-replete unmanipulated unit. Taken together, these

<table>
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<tr>
<th>Murine Double-Unit CBT</th>
<th>Single-Unit Dominance</th>
<th>Correlation with Engraftment in Patients</th>
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<tbody>
<tr>
<td>MNCs</td>
<td>18/21 (86%)</td>
<td>18/21 (86%)</td>
</tr>
<tr>
<td>CD34+ cells</td>
<td>1/11 (9%)</td>
<td>1/11 (9%)</td>
</tr>
<tr>
<td>CD34+ cells (plus CD34- cells from clinically engrafting unit)</td>
<td>9/9 (100%)</td>
<td>9/9 (100%): clinically engrafting unit engrafted</td>
</tr>
<tr>
<td>CD34+ cells (plus CD34- cells from clinically nonengrafting unit)</td>
<td>11/11 (100%) exclusively or predominantly nonengrafting unit</td>
<td>0/11 (0%): all engrafted with clinically nonengrafting unit</td>
</tr>
</tbody>
</table>

CBT = cord blood transplantation; MNCs = mononuclear cells.
Figure 26-4. Comparison of high-resolution donor/recipient HLA-matching of each unit of double-unit pairs in 79 patients with sustained donor engraftment: the HLA match of the unit to the recipient does not influence unit dominance. In this figure, the 10-allele HLA match of the dominant unit (left axis) of each double-unit pair is linked by a line to the 10-allele HLA match of the corresponding nondominant unit of that graft (right axis). Unit pairs in which a unit had the same unit/recipient HLA match are linked by dashed lines. Unit pairs with dominant units that were more closely HLA matched to the recipient than the nondominant unit of the pair are linked with solid lines. Linked with dotted lines are pairs in which dominant units were less closely HLA matched to the patient. The dominant unit is not necessarily more closely HLA matched to the patient than its nondominant unit partner. Individual lines may represent more than one double-unit pair.
findings suggest that unit dominance likely involves a complex interplay of hematopoietic potential, as suggested by the role of CD34+ cell viability, and immune factors. These immune factors are likely T-cell mediated, as no role for natural killer cells has been identified to date.23 Future studies need to elucidate the specific cell population mediating the graft-vs-graft effect in the setting of two units with adequate engraftment potential.

Determinants of the Speed and Success of Neutrophil Engraftment

Although the influence of cryopreserved TNCs and infused CD34+ cell dose on engraftment of single-unit CB allografts is well documented, relatively little is known about how cell dose influences double-unit CBT engraftment. This has been analyzed in the MSKCC series of 84 double-unit CBT recipients (Table 26-2).10 A high incidence of sustained donor neutrophil engraftment was seen: 93% in myeloablative transplant recipients at a median of 23 days (range = 12-43) and 96% in nonmyeloablative CBT recipients at a median of 9.5 days (range = 7-36). At 21 days after transplantation, marrow hematopoiesis was derived from a single unit in the majority of patients regardless of conditioning intensity: 53/61 (87%) recipients of myeloablative conditioning and 14/22 (64%) recipients of nonmyeloablative conditioning (including four of the five patients with clinical graft failure). However, in the patients with engraftment of both units, one unit dominated, and in nearly all patients engraftment of the dominant unit progressed such that sustained engraftment of both units was observed in only one patient by 1 year after transplantation at the time of the analysis. Interestingly, the detection of two CB units early after transplantation did not increase the speed or likelihood of donor-derived neutrophil recovery (p = 0.71). This is an important observation, as any possible advantage with double-unit CBT is not due to transient engraftment of the ultimately nonengrafting unit, but rather to improved conditioning and immunosuppression compared to that used in series of historical controls. This increases the chance
of infusing at least one unit of good quality, potential graft-vs-graft interactions, possible dose-dependent effects of the total T-cell dose\textsuperscript{10} (see below), or perhaps combinations of these factors.

In the Avery study, detailed analysis of the influence of infused TNC, CD34\(^+\) cell, CFU, and CD3\(^+\) cell dose on the speed and success of sustained donor engraftment was performed in the subset of 61 patients who received myeloablative conditioning (Table 26-2).\textsuperscript{10} Although the infused CD34\(^+\) cell and CFU dose had no effect on unit dominance, a higher CD34\(^+\) cell and CFU dose in the dominating unit was strongly associated with improved engraftment (\(p = 0.0008\) and \(<0.0001\), respectively). However, interestingly, a higher infused total (unit 1 plus unit 2) graft TNC and CD3\(^+\) cell dose also had a significant impact on engraftment, whereas the cell doses of the nondominant unit had little association with engraftment (TNC dose) or no effect (CD34\(^+\), CFU, CD3\(^+\) cell dose). It is possible that T cells, even if not contributing directly to sustained engraftment (as in the case of the nondominant unit), may afford a graft-facilitating effect in a dose-dependent manner. This hypothesis will need to be analyzed in larger series that would permit multivariate analyses. In the meantime, the authors’ demonstration of the critical importance of the dominant unit CD34\(^+\) cell and CFU doses suggests that as with single-unit CBT, progenitor cell dose remains a critical attribute of the graft.

The association between the dominant unit CD34\(^+\) cell dose and engraftment in the 61 myeloablative double-unit CBT recipients transplanted at MSKCC is shown in Fig 26-5. A dominant-unit infused CD34\(^+\) cell dose \(>2 \times 10^7/\text{kg}\) was associated with 100\% engraftment and a faster speed of neutrophil recovery at 16.5 days (\(p <0.001\)). By contrast, when the infused CD34\(^+\) cell dose of the engrafting unit was 1.0 to \(2.0 \times 10^7/\text{kg}\), all patients engrafted but at a delayed median of 20 days, and only 89\% of patients who received \(<1.0 \times 10^7/\text{kg}\) engrafted at a median of 27.5 days. The critical importance of the cell dose of the unit dominating in engraftment that was observed explains why series investigating the infusion of multiple (3-5) small CB units resulted in unfavorable engraftment rates and high mortality.\textsuperscript{24,25}

In contrast to the critical importance of cell dose, neither unit/recipient nor unit/unit HLA-match (six HLA loci or 10 HLA alleles) analyses have revealed any association with double-unit engraftment.\textsuperscript{10} However, this does not mean that the HLA match plays no role. The New York Blood Center demonstrated, in analysis of 1061 single-unit CBTs, that a better unit/recipient HLA match was associated with improved engraftment, lower graft-vs-host disease (GVHD) and TRM, and enhanced disease-free survival.\textsuperscript{4} It is likely that similar analyses of much larger numbers of double-unit CBT recipients will have similar findings.

**Practical Implications for Unit Selection**

The study of double-unit CBT has generated findings that have important implications for CB unit selection. First, the percentage of viable CD34\(^+\) cells is a critical measure of unit quality; low viability units are unlikely to engraft in the double-unit setting.\textsuperscript{16} This introduces unit quality as a third major unit selection criterion along with TNC dose and HLA match. Significantly, a variation in CD34\(^+\) cell viability has been identified not only between units but between banks, suggesting banking practices may influence unit quality and engraftment potential. From a practical standpoint, MSKCC determines CD34\(^+\) cell viability within 2 hours of thawing on transplant day and ensures that at least one unit with adequate viability, and thus engraftment potential, is infused. The unlikely event that both units have poor viability (CD34\(^+\) cell viability <75\%) would trigger the emergency shipment of a backup unit (or units) previously reserved in the domestic inventory. Whether CD34\(^+\) cell viability influences the likelihood of graft failure
Figure 26-5. Engrafting unit CD34+ cell dose predicts the speed and success of neutrophil engraftment (n = 61). (Adapted from Avery et al.10)
Double-Unit Cord Blood Engraftment

in single-unit CBT recipients has not yet been investigated, but such a relationship is likely given the correlation of CD34+ cell viability with CFUs and the influence of CFU dose on single-unit CB engraftment. In addition, although CD34+ cell viability is available on transplant day and thus has an advantage over CFU content, it still requires thawing of the unit. Ideally, the field should move toward the standardization of progenitor cell content measurement from an attached segment so that this could be used for unit selection before unit shipment.

A second major finding in double-unit CBT recipients is that the unit that is responsible for sustained donor engraftment cannot be predicted at the time of unit selection. Therefore, because the TNC dose and the even more critical CD34+ cell and CFU doses will predict the speed and success of engraftment, the cell dose of each unit is equally important and must be carefully considered for each unit. Furthermore, as stated above, although unit/recipient HLA matching has not yet been shown to influence double-unit engraftment, the available series is far too small to be able to make any definitive statement regarding engraftment; moreover, it is likely that the HLA match will be a critical determinant of GVHD and TRM after double-unit CBT as in single-unit CBT. However, because there appears to be no relationship between engraftment success and unit/unit HLA match, the authors have abandoned this criterion in unit selection at MSKCC, although the influence of unit/unit HLA match on transplant outcomes should continue to be studied. Dropping this requirement is significant because obeying an arbitrary unit/unit HLA-match rule could dictate the selection of a second unit for a double-unit pair that has an inferior TNC dose, which could adversely affect engraftment if this unit was dominant in engraftment.

Conclusions

Double-unit CBT has extended access to potentially curative allografts in that it allows patients to receive transplants when they would otherwise be excluded based on an inadequate single-unit graft. This is particularly important given that CBT can extend transplant access to racial and ethnic minorities. The understanding of double-unit engraftment biology is of scientific interest from the standpoints of both engraftment and potential graft-vs-malignancy effects. Unit dominance is likely a complex interplay of hematopoietic potential and immune phenomena, whereas the speed and success of engraftment is dictated by the CD34+ cell and CFU dose of the dominant unit, although the total graft TNC and CD3+ cell dose may also play a role. The routine use of double-unit CBT establishes it as a platform to test novel transplantation approaches such as ex-vivo expansion, for example. The routine use of cells from more than one donor has likely also increased the acceptance of the investigation of further novel cellular therapies, including third-party cells. Future investigation of the biology and practice of double-unit CBT should further advance the field of allogeneic stem cell transplantation.

References


23. Tarek N, Gallagher MM, Chou JF, et al. KIR-HLA genotypes have no identifiable role in unit predominance following double unit
cord blood transplantation. BBMT 2011;17: S286.