Umbilical cord blood transplantation: a maturing technology

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The use of umbilical cord blood (CB) as a source of hematopoietic progenitor cells for patients with high-risk hematologic disorders receiving allogeneic hematopoietic cell transplantations (HCTs) has increased significantly. Single-institution and registry studies have shown a decreased relapse rate and an increased transplantation-related mortality rate with similar overall survival rates after allogeneic HCT with CB compared with other donor sources. The transplantation of double CB units has overcome the dose limitation inherent in a single CB unit and thus has markedly extended the use of CB to larger children and adults. Similarly, the use of reduced intensity conditioning in the CB transplantation setting has allowed the treatment of older patients who would be unable to tolerate the myeloablative regimens used in the original CB transplantation protocols.

Introduction

In recent years, there has been exciting progress in the use of umbilical cord blood (CB)–derived immune cells in the hematopoietic cell transplantation (HCT) setting. There are now several strategies designed to reduce graft-versus-host disease (GVHD) and to improve CB homing, engraftment, and immune reconstitution. These novel approaches will likely change the current application of CB transplantation (CBT) with the goal of producing even better clinical outcomes.

Only 30% of patients who need allogeneic HCT have a matched sibling donor. The National Marrow Donor Program (NMDP), which was established in 1986, and its cooperative international registries have 16 million volunteer donors.1 It is estimated that 60% of white patients, but only 20%-45% of African-American and other minority patients, will be able to find a suitably matched unrelated donor (MUD) and proceed to transplantation. Therefore, there are an estimated 5000 patients per year who are candidates for alternative donor HCT. Their options include mismatched related (often haploidentical), CB, or mismatched unrelated donors (MMUD).

Since the first CBT was performed in 19892 to treat a child with Fanconi anemia, CB has emerged as an alternative source of hematopoietic stem cells.3 Expanding on the success in pediatric CBT pioneered by Drs Kurtzberg, Gluckman, Wagner, Broxmeyer, and others,4,5 the field grew rapidly. As of 2011, more than 25,000 CBTs have been performed worldwide and more than 500,000 CB units have been donated for public use. CB is readily available and donors can be found for diverse patient populations.6

The use of CB for HCT provides some potential advantages compared with the use of bone marrow (BM) or mobilized peripheral blood progenitor cells (PBPCs). Advantages include ease of collection, with little to no risk to the mother or newborn; prompt availability, with patients receiving CBT a median of 25-36 days earlier than those receiving unrelated BM; low risk of infection transmission; decreased stringency of human leukocyte antigen (HLA)–matching requirements; and the relatively lower risk of GVHD with preserved graft-versus-malignancy effects. In contrast to BM or PBPCs, which generally require a high degree of HLA match between donor and patient,8 this reduced incidence of GVHD with partially HLA-mismatched CB is likely due to the lower numbers of T cells and the relatively immunologically naive status of the lymphocytes in CB.9

CBT in pediatric patients

Although several studies have demonstrated a benefit of CBT in children with hematologic malignancies,10-15 the first prospective, multicenter trial of CBT in 191 pediatric patients with hematologic malignancies was reported by Kurtzberg et al on behalf of the Cord Blood Transplantation Study (COBLT).16 The overall survival (OS) of the study population, 77% of whom had high-risk disease, was 57.3% at 1 year. Those results were compared favorably with those published from registry and single-center data showing disease-free survival (DFS) of 50%-60% in those with early-stage disease and...
10%-30% in those with more advanced and active disease. A landmark study was reported by Eapen et al on behalf of The Center for International Blood and Marrow Transplant Research (CIBMTR).17 That study compared the outcomes of 503 children under the age of 16 years with acute leukemia who had received transplantation with HLA-matched (4-6/6) CB with outcomes of 282 HLA-matched (7-8/8) unrelated donor BM recipients. CBT compared favorably to the gold standard of 8/8 allele-matched unrelated BM transplantation, supporting the use of HLA-matched or HLA-mismatched CBT in children with high-risk acute leukemia without a matched related donor (MRD). The recipients of 1-antigen–mismatched CB units with a lower cell dose had engraftment similar to 2-antigen–mismatched units, whereas 1-antigen–mismatched CB units with a higher cell dose had superior engraftment, indicating that cell dose partially compensated for the degree of HLA mismatch.

There has been an increasing interest on the use of CBT for the treatment of metabolic diseases, hemoglobinopathies, and immune deficiencies in children.18-22 Kurtzberg et al has pioneered the use of CBT with very encouraging preliminary results in children with inherited metabolic disorders,18,23 including Krabbe disease and Hurler syndrome. Registry studies also showed acceptable outcomes after CBT in patients with severe combined immune deficiency compared with other donor sources.24

CBT in adults

The first large series of adults receiving CBT was reported by Laughlin et al in 2001.25 This analysis of 68 heavily pretreated patients with advanced hematologic malignancies demonstrated the feasibility of performing CBTs after myeloablative conditioning (MAC) in adult patients, with an event-free survival (EFS) of 26%. Similar to what had been described in pediatric series, a higher cryopreserved nucleated cell dose (≥ 2.4 × 10^7/kg) was associated with faster and a higher probability of neutrophil recovery and a higher cryopreserved CD34+ cell dose (≥ 1.2 × 10^5/kg) was associated with a better EFS rate in this groundbreaking study. Neither patient age nor HLA matching appeared to influence EFS. Cornetta et al subsequently reported a 30% 6-month survival rate for the COBLT prospective study of 34 adult patients (median age, 34 years) who received MAC for advanced malignancies.26 Over time, results of CBTs have been improving. Two large registry studies compared disease outcomes for adults after CB or unrelated BM transplantation with MAC. Rocha et al observed similar leukemia free survival (LFS), transplantation related mortality (TRM) and relapse incidence despite delay of engraftment in CB recipients with acute leukemia who received MAC compared with results in recipients of MUD transplantations.27 Laughlin et al observed higher TRM and shorter LFS with CB compared with 6 of 6 HLA-matched MUD, whereas the LFS was similar to 5/6 HLA-matched MUD.28 None of those studies compared CB with PBPC transplantations.

Recently, Eurocord and the CIBMTR performed a study comparing unrelated BM (n = 472) or PBPC (n = 888) transplantation with CB (n = 165) HCT in adults with acute leukemia.29 In that study, allele-level HLA typing was performed for the adult donors at A, B, C, and DRB1 loci and either 8/8 and 7/8 matched donors were included. All CB units were HLA-typed at the antigen level for the A and B loci, with allele-level typing for DRB1. CB units matched at 4-6/6 loci were included. CB recipients received a single unit containing a minimum of 2.5 × 10^7 total nucleated cells/kg of bodyweight at cryopreservation. Multiple regression analyses revealed higher TRM but lower relapse and GVHD rates with CB, leading to comparable LFS rates compared with other stem cell sources (Figure 2). The results of this study and others confirmed that CBT is feasible in adults when a CB unit has a higher number of cells and should be considered an option for allogeneic HCT in patients lacking an HLA-matched donor.

Double CBT

The relatively low number of progenitor cells present in a single CB unit resulting in delayed hematopoietic recovery and an increased rate of engraftment failure initially limited the use of CBT in adults. The majority of adults do not have access to a single CB unit containing the recommended nucleated cell dose of 2.5 × 10^7/kg.30 To overcome the cell-dose limitation, Barker and the University of Minnesota investigators pioneered the use of double CBT (dCBT), sequentially infusing 2 CB units instead of 1 after conditioning therapy.31,32 These investigators initially reported the safety and feasibility of dCBT in 21 adults with hematological malignancies after MAC HCT.31 The results were encouraging, with all patients engrafting neutrophils in a median of 23 days (range, 15-41). Interestingly, by day 21 in more than 80% of the patients, only 1 of the 2 CB units was detected and was responsible for the long-term hematopoiesis in those patients. In other studies, dominance of the “winning” unit as early as day 12 has also been reported.33,34
Ramirez et al showed that, in the myeloablative setting, CD3⁺ cell dose was the only factor associated with unit predominance, but in the nonmyeloablative setting, CD3⁺ cell dose and HLA match were independent factors associated with unit predominance. Although these findings suggest that immune reactivity may have a role in unit predominance, the biological mechanisms responsible for single-donor predominance after dCBT remain incompletely understood and are under investigation by many groups.

Another important observation after dCBT is the higher incidence of acute GVHD. MacMillan et al reported a higher rate of grade II-IV acute GVHD in recipients of dCBT (58%, n = 185) compared with those of single CB units (39%, n = 80) due to an increased rate of grade II skin GVHD. The rates of grade III-IV GVHD were the same for both groups and the 1-year TRM was significantly lower after dCBT compared with single CB (24% vs 39%).

Rodrigues et al reported a significantly lower risk of relapse at 1 year after dCBT compared with single CB in a study of 104 adult patients with lymphoid malignancies (13% vs 38%). Recently, a study prospectively comparing single CB versus dCBT in adult patients (assignment was based on the cell dose in the primary unit) confirmed previous findings of a lower relapse rate after dCBT versus single CB (30.4% vs 59.3%). The possible enhancement of graft-versus-malignancy may be due to greater alloreactivity when 2 CB units are infused, but this finding requires confirmation and further mechanistic studies.

The risks and benefits of dCBT (n = 128) relative to those observed after transplantations with MRD (n = 204), MUD (n = 152) or l-antigen-MMUD (n = 52) after MAC in leukemia patients were reported by the University of Minnesota in collaboration with the Fred Hutchinson Cancer Center. Risk of relapse was significantly lower in recipients of dCBT (15%) compared with MRD (43%), MUD (37%), and MMUD (35%) (Figure 3). However, TRM was higher for dCBT (24%) vs MRD (24%) and MUD (14%). LFS after dCBT was comparable to that observed after MRD and MUD transplantation, supporting the use of 2 partially HLA-matched CB units when patients lack an HLA-matched donor. Whether dCBT is preferable to single CBT when the cell dose in one unit is acceptable is not known. The ongoing Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) 0501 study (www.clinicaltrials.gov number NCT00412360) has randomized children with leukemia to single CBT versus dCBT for MAC HCT and the results are eagerly awaited.

**CBA after RIC regimens**

The development of reduced-intensity conditioning (RIC) regimens was particularly important in extending HCT transplantation to adults. Barker et al reported that the fludarabine, cyclophosphamide, and low-dose total body irradiation (TBI) regimen was well tolerated, with rapid neutrophil recovery, a sustained donor engraftment rate of 94%, and a low incidence of TRM. Rocha et al from the Eurocord Registry also reported similarly encouraging results using a RIC regimen of fludarabine, Endoxan, and TBI, with a 1-year

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**Figure 3. Clinical outcomes after myeloablative conditioning HCT by donor source.** Clinical outcomes after dCBT, MRD, MUD, and MMUD transplantation. (A) LFS. (B) Relapse. (C) Nonrelapse mortality. (D) Neutrophil engraftment. (E) Platelet engraftment. (F) Grade 2-4 acute GVHD. Reprinted with permission from Brunstein et al.39
Table 1. RIC regimens in CBT

<table>
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<tr>
<th>Reference</th>
<th>N</th>
<th>Disease</th>
<th>Conditioning regimen</th>
<th>Median age, y</th>
<th>Median time to ANC &gt; 500/µL, d</th>
<th>Grade II-IV acute GVHD</th>
<th>Chronic GVHD</th>
<th>TRM at d 100</th>
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<td>Hematological malignancies</td>
<td>Bu/Flu/TBI</td>
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<td></td>
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<td>Cy/Flu/TBI</td>
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<td>9.5</td>
<td>27%</td>
<td>23%</td>
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<td>33% at 1 y</td>
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<td>Flu/Me1/2-4 Gy TBI</td>
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<td>18</td>
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<td>Flu/Me1/4 Gy TBI</td>
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<td>20</td>
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<td>Hematological malignancies</td>
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<td>Bu/Flu/ATG Cy/VP16/ATG</td>
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<td>18</td>
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<td>60% at 5 y</td>
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<td>40%</td>
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<td>13%</td>
<td>13%</td>
<td>53% at 2 y</td>
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<td>61%</td>
<td>40%</td>
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<td>25</td>
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<td>Cy/Flu/TBI ± ATG</td>
<td>55</td>
<td>9</td>
<td>50%</td>
<td>34%</td>
<td>19 at 2 y</td>
<td>37% at 2 y</td>
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ANC indicates absolute neutrophil count; Bu, busulfan; Flu, fludarabine; Gy, Gray; Mel, melphalan; Cy, cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; VP16, etoposide; CB, cord blood; TRM, transplant-related mortality; OS, overall survival; and NA, not applicable.*The results were presented for the whole group.†Reported only grade III-IV acute GVHD.
transplantation. These results provide the rationale for a multina-
tional randomized trial beginning soon that will compare unmanip-
ulated dCBT with dCBT in which one of the units is expanded in
MSC cocultures.

Similarly promising results have been reported by Delaney et al
using Notch-mediated ex vivo expansion system for human CD34+
CB progenitors, with a decrease in the median time to neutrophil
recovery by more than 1 week compared with results reported after
infusion of 2 unmanipulated units.59 The expanded cells contributed
almost exclusively to initial myeloid engraftment observed at
1 week, demonstrating an enhanced capacity of the expanded cell
graft to provide rapid myeloid recovery. Furthermore, all but one
evaluable subject engrafted before day 21 regardless of whether the
expanded cell graft persisted in vivo.

A pivotal trial sponsored by Gamida Cell Ltd evaluated the ex vivo
expansion of a fraction of a single CB unit using growth factors in
conjunction with the copper chelator tetraethylpentamine.60
Preliminary analysis revealed faster engraftment and improved
survival compared with historical control recipients of single CB
reported to the international registries.51 A more definitive analysis
of these data is in progress. In preclinical studies, CB CD34+ cells
cultured ex vivo with growth factors (SCF, thrombopoietin, IL-6,
and FMS-related tyrosine kinase 3) and nicotinamide (pyridine-3-
carboxamide) displayed increased migration toward SDF-1 and
enhanced homing to BM compared with untreated CB.61 A pilot
clinical trial led by Horwitz et al is in progress to evaluate this
strategy in the dCBT setting (www.clinicaltrials.gov number
NCT01221857). Preliminary analysis has shown rapid engraftment
(10 days for neutrophils and 30 days for platelets) with, interest-
ingly, sustained engraftment coming from the expanded unit in the
majority of patients evaluated to date (personal communication with
Dr Horwitz).

Improving CB homing to BM

Another strategy to improve engraftment is to correct the decreased
fucosylation of CB cell-surface molecules, which is thought to
impair homing of CB-derived progenitor cells to the BM.62 In
murine models, Robinson et al showed that CB CD34+ cells treated
with fucosyltransferase-VI led to more rapid and higher levels of
engraftment.63 A clinical trial in which recipients will receive dCBT
with fucosyltransferase-VI led to more rapid and higher levels of
treatment IL-2 is being conducted by the University of Minnesota
investigators in refractory AML patients to investigate NK-cell
expansion and function in vivo (www.clinicaltrials.gov number
NCT01464359).

CB-derived Tregs

Regulatory T cells (Tregs) are a subset of CD4+ T cells that
coopress CD25 (IL-2Rα chain) and high levels of Foxp337 and are
dependent on IL-2. Tregs represent a novel cell-based approach for
potentially reducing the risk of GVHD.

Brunstein et al expanded Tregs obtained from a third CB unit and
infused them into 23 patients undergoing dCBT.72 No severe
Treg-related acute toxicities were observed and accrual to the study
continues, with refinements in the Treg-generation procedure.

Conclusions

CB is used increasingly as a source of allogeneic hematopoietic
support for patients who need HCT and do not have access to an
HLA-matched donor. To overcome the limitation of low cell doses
in single CB units, dCBT has been adopted for many patients and is
associated with outcomes comparable to those with other donor
sources. There have been new strategies under development to
improve engraftment with ex vivo expansion or homing and to
enhance immune reconstitution with the infusion of CB-derived NK
cells and cytotoxic T lymphocytes with antiviral and antileukemic
specificities. Tregs are being evaluated to reduce the incidence of
GVHD. Prospective, multicenter clinical trials are needed to
determine the efficacy of these promising technologies that are
likely to improve outcome for CBT patients.
Disclosures
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