
Cord Blood: an Alternative Stem Cell Source or a New Standard?

Juliet N. BARKER

Memorial Sloan-Kettering Cancer Center, NY, ABD

In contrast to the very high transplant-related mortality (TRM) associated with the early experience of cord blood (CB) transplantation (CBT), recent transplant series have been associated with comparable survival to that of human leukocyte antigen (HLA)-matched unrelated donor transplantation in children, with similarly promising results in adults. Consequently, the use of CB as an alternative stem cell source and the global inventory of units in public banks are rapidly increasing. While challenges remain, the optimization of CB transplantation (CBT) can permit CBT to be a standard alternative hematopoietic stem cell (HSC) source.

CBT Compared to Unrelated Donor Transplantation

Since the first CBT from an HLA-identical sibling 21 years ago progress in unrelated CBT has been paralleled by the development of CB banks worldwide. Today, at least 350,000 CB grafts are available in more than 45 banks and more than 14,000 CBT have been performed. CB can reconstitute hematopoiesis in adults following both myeloablative¹⁻⁷ and reduced-intensity (RI)/ non-myeloablative (NMA)⁸⁻¹⁰ conditioning. In contrast to allogeneic HSC transplantation using volunteer donors, CB has the advantage of ready availability and a reduced HLA-match requirement^{1-3,11}. Recent unpublished MSKCC data suggests that CB therefore extends transplant access to racial and ethnic minorities. In addition to the less than expected incidence of graft-versus-host disease (GVHD)^{1-3,11}, the GVHD after CBT may be easier to treat¹².

While no randomized controlled trials have compared CBT and unrelated donor (URD) transplantation, retrospective studies have compared

single unit CBT with URD bone marrow (BM) transplantation (BMT) using myeloablative conditioning in adults and children. In 2004 Laughlin et al⁴ and Rocha et al⁵ reported the first comparisons between CBT and URD transplantation in adults. The American series found comparable survival after UCBT (n=150) and 1 antigen mismatched BMT (n=83)⁴ whereas the Europeans reported that HLA-mismatched adult CBT (n=98) was associated with comparable survival to 6/6 HLA-antigen matched BMT (n=584)⁵. In contrast, a Japanese series reported by Takahashi et al demonstrated superior TRM and disease-free survival (DFS) in 68 adult CBT as compared to 45 URD BMT recipients⁶.

More recently, Eapen et al have analyzed the outcomes of 503 CBT recipients of 4-6/6 HLA-A, B antigen and DRB1 allele-matched single unit UCBT as compared to those of URD BMT in children <16 years of age with leukemia¹³. Most notably, in a subset analysis comparing CBT outcomes with the 116 recipients of the "gold standard" of 8/8 HLA-allele matched BM, the 35 6/6 HLA-matched CBT recipients had significantly higher 5 year DFS, with 201 5/6 and 267 4/6 CBT having comparable DFS with that of 8/8 allele matched BMT recipients and demonstrated a robust protection against relapse.

These findings support CBT as an alternative to URD BMT in children. Further, if engraftment after CBT is improved, it suggests that pediatric CBT may be a superior HSC for the treatment of leukemia. In adults, the American and European comparisons, while establishing CBT as a potential alternative to URD BMT, have highlighted that the poor engraftment and high TRM must be addressed for this HSC to be widely adopted. At

this time whether CBT will be offered to a patient is frequently determined by: 1) the relative availability of a closely HLA-matched (7-8/8 alleles) URD versus a CB graft of $\geq 4/6$ HLA-A,B antigen and DRB1 allele match and adequate dose; and 2) the experience and research bias of the transplant center. Further, to fulfill the promise of CBT it is important to consider all strategies by which CBT outcome can be optimized.

When to Consider CB as a HSC Source

The first step to optimize CBT outcome is to consider CB as a potential alternative as soon as the patient is an allogeneic transplant candidate but does not have any suitable sibling donors and before the disease is far advanced. An URD HSC algorithm must be established that states how much HLA-mismatch will be tolerated in HLA-A,B,C,DRB1,DQ allele typed URD before an alternate HSC source is sought. Prolonged URD searches can compromise the patient's care. Therefore, the patient's HLA-typing, the preliminary URD search, and the patient's ancestry should be reviewed to assess the likelihood that a suitably matched URD will be secured in the required time period. For patients with less common HLA-typing, especially non-Europeans, a simultaneous CB search should be performed.

The CB Search

The CB search continues to be a challenge with no centralized search mechanism to access all units in the global inventory. Standardizing banking standards including the information needed by TCs should be a priority. In the meantime to assist search coordinators the TC should establish: 1) what banks will be searched; 2) a unit selection algorithm that defines a satisfactory single unit; 3) criteria for a double unit graft; and 4) whether back-up units will be reserved in the event there are problems with shipping/thaw or graft failure. Factors to be considered in unit selection are summarized in Table 1.

The Preparative Regimen and Immune Suppression (IS)

While the ideal conditioning for CBT is not defined, it is likely changes in conditioning and IS can improve CBT outcome. For example, consider the improved survival reported with myeloablative double unit CBT¹⁴. The single unit historical controls were transplanted using cyclophosphamide (Cy) and total body irradiation (TBI) with

anti-thymocyte globulin (ATG) and cyclosporine-A (CSA)/ methylprednisolone (MP)³. While the double unit transplants were also performed with Cy/TBI+CSA, the ATG and MP were substituted with fludarabine (Flu) and mycophenolate mofetil (MMF) possibly accounting for some of the improvement. This question is therefore being investigated in the Clinical Trials Network single versus double unit randomized trial in children utilizing Cy/Flu/TBI+CSA/MMF in the US.

For patients that cannot tolerate high-dose conditioning reduced-intensity (RI) or non-myeloablative (NMA) conditioning in patients with high-risk hematologic malignancies has been associated with an overall survival (OS) of 45% and progression-free survival of 38% at 3 years⁹. A major question in NMA CBT is how to ensure engraftment in patients without recent exposure to combination chemotherapy or a prior autologous transplant given the addition of ATG has been associated with a high incidence of post-transplant lymphoproliferative disease¹⁵. Investigation of preparative regimens that include agents that augment recipient IS without impacting the graft should be a priority in this patient population. Further, the efficacy of RI/ NMA CBT in specific diseases should now be the subject of Phase II studies.

While the ideal prophylaxis against GVHD in CBT has also not been established CSA/MMF is relatively well tolerated in patients with intact renal function. Methotrexate should likely be avoided due to the risk of delayed engraftment. The use of ATG is associated with impaired immune recovery^{15,16} and corticosteroids should likely similarly be avoided. Whether tacrolimus/ sirolimus may be another promising alternative is yet to be established.

The CB Graft

Graft failure is a major risk associated with CBT and from early in CBT experience it was recognized that the total nucleated cell (TNC) dose and the infused CD34+ dose/kilogram were significant determinants of sustained donor engraftment. Perhaps the simplest strategy being investigated to augment engraftment is the infusion of a double unit graft. This approach is as equally relevant to many children as adults given graft failure remains a devastating feature of many pediatric series and many larger children will only have access to units of relatively low cell dose.

Initial investigation with myeloablative double unit CBT yielded a DFS of 57%¹⁴ with updated analysis of 83 patients with high-risk hematologic malignancies showing a DFS of 54% (personal communication, Professor John Wagner). Engraftment appears improved after double unit CBT despite only one relatively low cell dose unit engrafting suggesting that the “losing” unit is facilitating the engraftment of the winner. Interestingly, analysis of post-thaw CD34+ cell viability has suggested that double unit CBT is efficacious because it increases the chance of receiving at least one unit of high viability and thus with engraftment potential¹⁷. This suggests that post-thaw CD34+ cell viability could be an effective measure of unit quality and, unlike colony-forming unit (CFU) assays, is available on transplant day. Methods to determine unit quality both prior to and at thaw should be a major priority.

While the poor engraftment and high TRM associated with low TNC dose in single unit CBT has led to a focus on graft cell dose, unit selection is complicated by HLA-match also influencing engraftment and TRM. For example, in an analysis of 989 single unit myeloablative CBT recipients facilitated by the New York Blood Center (NYBC), HLA-A,B antigen, DRB1 allele match was associated with significantly improved engraftment, less severe acute GVHD, lower TRM, and improved survival¹⁸. Therefore, a critical decision is between a smaller better matched versus the larger less matched unit. The NYBC analysis suggests that HLA-match can partially compensate for lesser cell dose with a selection algorithm of 6/6 units, 5/6s $>2.5 \times 10^7$ /kg, and 4/6s $>5.0 \times 10^7$ /kg. However, many patients will not have access to such units, and some with

such units will still not engraft. Clearly, more work is needed to resolve the dose versus match “trade off”.

The Thaw

The albumin-dextran dilution with centrifugation (“wash”) thaw methodology is appropriate for small children but has been adopted for adult CBT as a matter of convention. However, a “no wash” technique dilutes the product without centrifugation and is advantageous for adolescents and adults in whom even modest cell losses may be significant. This technique is faster, more efficient, reduces unit manipulation, speeds time to infusion, and reduces the potential for cell loss and is still done in the controlled laboratory environment. This thaw has been adopted for patients over 20 kg at MSKCC for red blood cell depleted units, and in 54 double unit CBT recipients has been associated with no serious infusion reactions and sustained donor engraftment in 94% of patients and survival of 65% at one year post-transplant¹⁹.

GVHD, Infection and Relapse

CBT has consistently demonstrated a lower than expected incidence of acute and chronic GVHD^{1-3,11}. However, CB can be associated with severe GVHD and transplant success is contingent upon therapeutic levels of a calcineurin inhibitor (CI) in the first months after transplant, and how to transplant patients who cannot tolerate CI is not established. The nature of acute GVHD after CBT and its response to therapy has yet to be examined in detail. However, Arora et al found more frequent therapy responses and a lower TRM in CBT recipients with chronic GVHD as compared to pre-

Table 1. Major Criteria for Cord Blood Unit Selection

<i>Criteria</i>	<i>Comments</i>
• HLA-A,B antigen, DRB1 allele match	<ul style="list-style-type: none"> • While HLA-match requirement less stringent, HLA-match still a critical determinant of TRM. • Up to 2 mismatches acceptable although how to trade off TNC dose and HLA-match not known.
• TNC dose/kg	<ul style="list-style-type: none"> • Threshold for acceptable cell dose likely varies according to HLA-match (the more mismatch the higher the required TNC). • Use units $<2.5 \times 10^7$/kg with caution. • Need to correct TNC of RBC replete units in order to compare to TNC of RBC depleted units.
• Bank of origin	<ul style="list-style-type: none"> • Quality of units can vary from unit to unit and bank to bank. • Speed of turnaround time, reliability of unit information, and testing fees can vary from bank to bank.
• CT from attached segment	<ul style="list-style-type: none"> • Only way to confirm unit identity.
• IDMs and hemoglobinopathy testing	<ul style="list-style-type: none"> • Should ensure completeness of testing so as not to slow down acquisition of the unit. Must be completed before unit is shipped.

HLA=human leucocyte antigen; TNC=total nucleated cell; RBC=red blood cell; CT=confirmatory HLA typing; IDMs=infectious disease markers.

dominantly HLA-matched URD BMT recipients¹². It is possible that some CBT recipients with GVHD may be successfully treated with less IS than is traditionally administered as therapy after adult donor transplants. This is another field requiring investigation in CBT.

Infection is a major challenge after CBT and at many centers infection-related mortality is the most frequent cause of CBT death with the majority of deaths occurring within the first 3-4 months. While aggressive supportive care to abrogate neutropenic sepsis and prevent fungal infections with extended spectrum azoles have led to decreased TRM, viral infections such as cytomegalovirus (CMV) or adenovirus remain a critical challenge in the early post-engraftment period²⁰. How to augment immune recovery is a major question. Cellular therapy approaches to the treatment of both infection and relapse, while challenging given the naïve neonatal immune system, may yet show promise. In the interim, avoidance of ATG as well as aggressive supportive care including surveillance for viral reactivation is critical in early post-transplant. In regard to relapse, the single best strategy to prevent this complication is to refer the patient for transplant before disease is advanced or refractory. Measures to augment immune reconstitution assume greater importance given improved immune recovery has been associated with protection against leukemic relapse²¹. Interestingly, preliminary data has suggested that double unit CBT may be associated with a reduced relapse risk^{9,22,23}.

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