
Strategies for widening the use of cord blood in hematopoietic stem cell transplantation

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The advantages of umbilical cord blood (CB) in hematopoietic transplantation are well known, and include the lack of risk to the donor, the rapid and easy procurement process and the low likelihood of transmitting infectious diseases. Furthermore, the increased tolerance to donor-recipient human leukocyte antigen (HLA) mismatches makes this source of hematopoietic stem cells (HSC) a very important alternative for patients without HLA-matched donors. If HLA mismatches can be tolerated, the donor pool is thereby increased, directly benefiting minority populations currently unlikely to have a bone marrow or peripheral blood stem cell donor given more stringent matching cri-

teria. Potential limitations of CB include the inability of predicting the development of future donor diseases that could affect the recipient, and the impossibility of obtaining further HSC or lymphocytes for the treatment of engraftment failure or disease relapse after transplantation. As the number of adult long-term survivors increase, the effects of delayed immune recovery and chronic graft-versus-host disease (GVHD) are becoming more prevalent. However, the major obstacle to the widespread use of CB in HSC therapy is the low cell dose available for transplantation.¹ A correlation between the number of CB mononuclear cells (MNC) transplanted per kilogram (kg) body weight and time to

engraftment, suggests that patients >45 kg receiving a single CB unit will have markedly prolonged times to engraftment (as measured by neutropenia and thrombocytopenia) and higher rates of engraftment failure, infectious complications and early treatment-related mortality. As a consequence, the majority of patients receiving successful CB transplantation to date have been children (average weight 20 kg).

Total nucleated cell (TNC) doses in the range of 1×10^7 per recipient kg are associated with high rates of early mortality, generally ascribed to the protracted time to myeloid recovery. Cell doses greater than 2.5×10^7 per kg are linked with lower rates of early mortality, but large individuals are much less likely to have such cell doses available. In selected Cord Blood Banks in the United States, approximately 38% and 24% of the banked units contain 1×10^7 total nucleated cells per kg for adults weighing 80 and 100 kg, respectively. If the cell dose to be used for transplantation is 3.7×10^7 TNC/kg, as recommended by Gluckman *et al.* for optimal survival² only 0.2% and 0.1% of the units would be available for adults in that weight range.

How does CB compare to other sources of HSC for transplantation of patients without HLA-compatible donors in the family? A key issue in any comparison, which is difficult to address, is the timing of transplantation and delays imposed by unrelated bone marrow or peripheral blood searches. Here, CB should provide a major advantage. However, most patients are treated with CB transplants after a search for an unrelated donor has failed, and are very often in a more advanced stage of disease. Search delays may also be seen as a bias in favor of those patients in remission undergoing unrelated transplants in general: the disease was somehow kept in remission long enough for the patient to make it to transplantation. These and other biases are unfortunately unavoidable in a scenario in which a prospective randomized trial of different stem sources is unlikely to be available in the near future. Furthermore, the availability of haploidentical donors adds another question to this already complex situation.

Rocha *et al.* reported a retrospective analysis of registry data in Europe comparing outcomes of patients with acute leukemia treated with unrelated bone marrow versus CB transplants. The former group was older (32 versus 24 years), while the latter had more advanced disease (52% versus 33%). HLA matching was determined by low resolution typing at the A and B loci, with high-resolution typing at HLA-DRB1. Marrow recipients received fully matched products while 94% of CB transplants were mismatched in at least one locus. Acute GVHD rates were lower after CB transplants, while chronic GVHD rates were similar. As expected, neutrophil recovery was delayed in the CB subgroup (treated with a median of 2.3×10^7 TNC, as opposed to the more than one log higher dose of 2.9×10^8 TNC/kg in the bone marrow transplant subgroup), but treatment-related mortality and leukemia-free survival rates were comparable.²

The International Bone Marrow Transplant Registry performed a retrospective analysis of adult leukemia patients treated with transplants from matched unrelat-

ed donors, one-antigen mismatched unrelated donor marrow, or unrelated CB transplants. The authors reported that the matched marrow group performed better than the other study groups. However, recipients of mismatched marrow or mismatched CB transplants had similar rates of treatment-related and overall mortality. Interestingly, use of CB grafts with one or two mismatches produced similar outcomes.³

Low-resolution class I typing (HLA-A and -B) has been employed in the majority of published series of unrelated CB transplants. It is unclear to what extent high-resolution typing and matching will influence the results, as they have in the unrelated bone marrow transplant setting. Likewise, it is unknown whether matching (or added mismatches) at other loci such as HLA-C will improve outcomes. It is to be expected that use of units with the smallest possible number of mismatches will have a positive impact on GVHD and rejection rates, for example. These data will be forthcoming in the next several years as more patients receive CB transplants and more sophisticated typing of CB units is performed.

The multicenter prospective American COBALT study reported a day 100 survival probability of 0.47, with approximately 17% of the adult patients receiving unrelated donor CB transplants being alive after 12 months.⁴ In contrast, significantly lower early mortality rates have been described by several single center studies. Although these single center results may indicate the influence of growing experience with this source of stem cells, one cannot underestimate the effect of patient selection on outcomes.

Two approaches have been taken to overcome the cell dose barrier and thereby improve the feasibility of CB transplantation for adults. One has been to increase the total number of CB cells transplanted by the transplantation of multiple units. The second has been the use of *ex vivo* CB expansion. In this issue of the journal, Magro *et al.* from the Universidad Autonoma de Madrid propose another method, expanding the group's previous observations.^{5,6} They used CD34 or CD133-selected peripheral blood HSC obtained from a third party donor that were co-infused with one unrelated donor CB unit. Third party donors included mothers, other haploidentical relatives and, in a minority of cases, donors who did not share any haplotypes with the recipient.

Magro *et al.* treated a cohort of 27 patients composed mostly of young adults, likely more capable of withstanding the ablative preparative regimen and other transplant-associated toxicities. Patients had hematologic malignancies, a median age of 29 years and a median weight of 67 kg. There was some heterogeneity in the way patients were conditioned. Preparative regimens were based on total-body irradiation (TBI) in 22 cases, busulfan in four cases, and a combination of chemotherapy agents and low-dose TBI in one case. Changes were made in order to minimize regimen-related toxicity, and included a decrease in the radiation dose from 12 Gy to 10 Gy, and addition of fludarabine to maximize immunosuppression. Anti-thymocyte globulin (ATG) was used in all but two cases; one of the patients treated without ATG developed severe GVHD and the drug was reintroduced in subsequent patients.

The median time to CB neutrophil engraftment was 21 days, with the remarkable observation that the third party cells initially engrafted and were then progressively replaced by the CB cells, minimizing neutropenia-related problems, without an apparent increase in GVHD rates. Most importantly, 1-year non-relapse mortality was in the range of 20-25%. The observation that recipients of maternal third party donor cells had poorer outcomes than the recipients of non-maternal third party cells is interesting, but the small number of cases precludes any definitive conclusions at this point.

Even more intriguing was the successful use of third party donors that did not share any haplotype with the recipient. This observation, if confirmed in a larger number of cases, would significantly facilitate and expand the applicability of this strategy. It also serves as further *proof of principle* of the expectation that the third party cells were to be rejected progressively by the CB cells. One would hypothesize that donor cells that did not share any haplotypes with the recipient would have an even lower likelihood of long-term engraftment. What is the mechanism of rejection of third party cells? The data support the existence of a *graft-versus-graft* effect, at least with the low dose of third party T lymphocytes used, favoring the CB graft. Theoretically, recipient cells could also be involved to some extent in the rejection of third party donor cells. Mathe proposed in the 1960s that a phenomenon of *multiple tolerization* may operate on recipients of multiple grafts, leading to less GVHD.⁷ Is *multiple tolerization* a possible extra benefit of this combined transplantation approach? Such promising results require further investigation of the immune process operative early after this form of double transplant. Regardless of the mechanism, however, CB stem cells prevailed and provided long-term engraftment, with acceptable GVHD and infectious rates.

The process of CD34 or CD133 selection of the third party grafts adds another logistic step and financial burden to the transplant procedure, and it remains to be seen how applicable and reproducible this approach will be in a multi-institutional situation, or how it will compare to other experimental strategies under investigation.

Double CB transplants are another strategy under investigation to improve the outcome of adult CB transplantation. In the University of Minnesota experience, the median time to neutrophil engraftment was 23 days, while the acute grade III-IV GVHD rate was 13%, with a 57% 1-year disease-free survival.^{8,9} Interestingly, one unit prevails, and the vast majority of the recipients do not have sustained evidence of double chimerism. Unfortunately, there are no reported controlled studies comparing double versus single CB transplants.

Several groups, including ours, are interested in the *ex vivo* expansion of CB cells. While a major focus of expansion is directed at providing optimal numbers of HSC for transplantation, the generation and transplantation of more lineage-committed hematopoietic progenitors to abrogate chemotherapy-induced pancytopenia is also an important goal. Liquid culture expansion requires that CD133⁺, or CD34⁺ cells be isolated from CB prior to culture, as unfractionated CB does not expand well. Components of the growth factor cocktails used in *ex vivo*

HSC expansion protocols include: stem cell factor (SCF), interleukin (IL)-3, IL-6 and granulocyte colony-stimulating factor (G-CSF); SCF, thrombopoietin (TPO) and G-CSF; and Flt-3 ligand (FL), SCF, IL-3, IL-6 and G-CSF. FL and TPO appear to be important in supporting the self-renewal of primitive stem cells, possibly by preventing telomere degradation with proliferation, while SCF and IL-6 possibly enhance the proliferative potential of specific HSC subpopulations.

In a study of 37 patients, Shpall *et al.* demonstrated the efficacy of *ex vivo* expansion of isolated CD34⁺ CB cells.¹⁰ A fraction of the CB unit was expanded in the presence of SCF, TPO and G-CSF for 10 days. The TNC number increased by 56-fold and the total number of CD34⁺ cells by 4-fold. McNiece *et al.* subsequently developed a two-step, 14-day expansion protocol which appears to be more effective than the single-step 10-day protocol described above.¹¹ This two-step *ex vivo* expansion protocol yields a >400-fold increase in TNC and a >20-fold increase in CD34⁺ cells. A clinical trial employing this procedure is ongoing in our institution, where we are randomizing patients with high-risk hematologic malignancies between double unmanipulated CB transplants versus co-transplantation of an unmanipulated unit and a second unit that is 100% expanded *ex vivo*. Further modifications to this liquid *ex vivo* expansion technique have included, or may include in the future, the development of serum-free culture systems and the use of tetraethylenepentamine (TEPA), a copper-chelator thought to modulate the proliferation and differentiation of primitive hematopoietic progenitors.¹² Another approach is under investigation by Delaney *et al.* using the Notch ligand, Delta1, a known regulator of cell fate determination.¹³ Culture with an engineered Notch ligand form consisting of the extracellular domain of Delta1 fused to the Fc domain of human IgG1 (Delta1^{ext-16c}) led to a substantial increase in the number of CD34⁺ cells and in the rate and magnitude of repopulation in immunodeficient mice.

Ex vivo liquid culture expansion removes the HSC from the regulation and support provided by the stromal microenvironment, such that the cells rely entirely on the addition of an exogenous cocktail of growth factors to prevent apoptosis and stimulate proliferation (potentially driving differentiation at the expense of self-renewal). Mesenchymal stem cells (MSC) may provide a supporting stroma and be an alternative (co-culture) strategy for CB expansion. Allogeneic third party MSC have been shown to promote engraftment of CB CD34⁺ cells in NOD/SCID mice when co-administered¹⁴ and to have immunomodulatory activity.¹⁵ Co-culture of CB with MSC may better preserve (and possibly expand) a component of the more primitive, long-term repopulating cells, while also producing increased numbers of more lineage-committed, shorter-term repopulating cells. One advantage of the HSC-MSC co-culture technique is that no isolation of the CB HSC is required prior to expansion, minimizing loss of HSC.

As for the choice of an unrelated CB versus a haploidentical graft, we are again confronted with the reality that a randomized trial is not available to help treating physicians and their patients in the decision-making

process. Haploidentical relatives are widely available, but in order to minimize the risk of GVHD, some form of T-cell depletion is necessary, adding a complicating step to the transplantation process. Preliminary registry data have been reported comparing these sources of hematopoietic stem cells. Rocha *et al.* reported, on behalf of the European Group for Blood and Marrow Transplantation, a non-randomized, registration study comparing outcomes of patients with acute lymphocytic (ALL) or myeloid leukemias (AML), treated with either with unrelated CB or haploidentical transplants.¹⁶ CB recipients had slower neutrophil recovery and a higher incidence of grade II-IV acute GVHD. Patients with AML had similar transplant-related mortality, relapse and 2-year disease-free survival rates, regardless of the source of stem cells. Patients with ALL, however, had a significantly better outcome after CB transplant (36% versus 13%). This result was mainly due to a lower relapse rate among CB recipients when compared to the haploidentical cohort, suggesting that CB may be a better choice for patients with ALL without a matched family donor. Interestingly, the largest subgroup of patients treated by Magro *et al.* consisted of ALL patients (n=14).

Cost and reproducibility are major issues related to all the experimental approaches reviewed here, which will need prospective evaluation. In addition, comparisons of some of the techniques will require co-operation and willingness by several centers to address specific questions. The article by Magro *et al.* raises important issues and indicates the direction to another avenue of investigation in the field of CB transplantation. Shortening the time to engraftment should reduce the morbidity and mortality of CB transplantation: how this is going to be accomplished is an open question.

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