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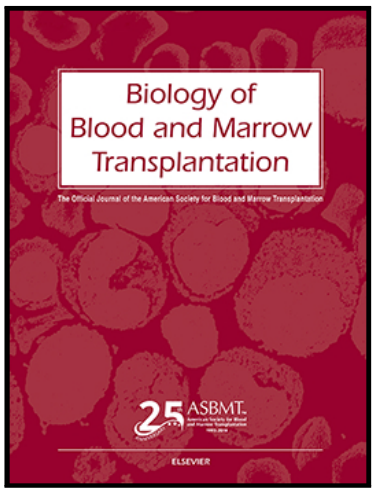

Guidelines for Cord Blood Unit Selection

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Guidelines for Cord Blood Unit Selection

Ioannis Politikos[1], Eric Davis[1], Melissa Nhaissi[1], John E. Wagner[2], Claudio G. Brunstein[3], Sandra Cohen[4], Elizabeth J. Shpall[5], Filippo Milano[6], Andromachi Scaradavou[7], Juliet N. Barker[1]

on behalf of the American Society for Transplantation and Cellular Therapy Cord Blood Special Interest Group

[1]Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

[2]Blood and Marrow Transplant Program, Department of Pediatrics, University of Minnesota, Minneapolis, MN

[3]Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, MN

[4]Division of Hematology, Department of Medicine, Maisonneuve-Rosemont Hospital, Montreal, QC

[5]Department of Stem Cell Transplant and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, TX

[6]Clinical Research Division, Fred Hutchinson Cancer Research Center & Department of Medicine, University of Washington, Seattle, WA

[7] Stem Cell Transplantation and Cellular Therapies, MSK Kids, New York, NY

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Corresponding author:
Ioannis Politikos, MD
Adult BMT Service, Department of Medicine
Memorial Sloan Kettering Cancer Center
545 E 73rd st, Box 61, New York, NY 10021
Phone: 646-608-2128; Fax: 929-321-8170
Email: politiki@mskcc.org

Background

Optimal cord blood (CB) unit selection is critical to maximize the likelihood of successful engraftment and survival after CB transplantation (CBT). Greater availability of high cell content quality units has likely contributed to improving CBT outcomes in recent years[1-6]. However, unit selection can be complex because multiple characteristics must be considered. Several reports have previously outlined country and transplant center-specific selection guidelines[7-12]. This review takes a frequently asked question (FAQ) approach to provide evidence-based guidelines for unit selection and experience-based recommendations when evidence is lacking. Additionally, a step-by-step unit selection guide is provided to simplify the process of performing searches and selecting CB grafts (**Table 1**).

FAQ1: What unit characteristics must be considered in CB graft selection?

Expert centers do not have a uniform approach to unit selection but agree upon the following principles:

1) Pre-cryopreservation total nucleated cell (TNC) *and* CD34+ cell doses must be considered.
2) Selection should be based on high-resolution 8-allele donor-recipient HLA-match.
3) Selection should be restricted to units of adequate quality.

FAQ2: How should CB unit cell dose be evaluated?

While the importance of TNC dose in CBT is well established, CD34+ cell dose is the most reliable predictor of engraftment[13-17]. Consequently, the current standard is to consider TNC *and* CD34+ cell doses in unit selection[7-11]. Consideration of CD34+ cell dose is essential because the TNC and CD34+ cell contents of banked units are not strongly correlated[18] and, consequently, units with an adequate or even high TNC dose may have intermediate or low CD34+ cell content[18, 19]. Consideration of TNC dose must remain, however, due to potential inter-laboratory variability and lack of standardization of CD34+ cell enumeration assays[20]. Rarely, units may be listed with unexpectedly high CD34+ cell content, and exclusion of erroneous data entry for such units is recommended. The CD34+ cell to TNC content (CD34+/TNC) ratio can be used to identify "out-of-range" CD34+ cell values that should be confirmed before final graft selection. An expected median CD34+/TNC ratio of 0.34% (IQR: 0.23-0.48) has been reported in an analysis of the U.S. inventory[18].However, a higher ratio of 0.78% (IQR: 0.6-1.07) has been observed in units selected for transplantation when CD34+ cell dose is also considered in unit selection (*Politikos I. et al, unpublished 2020[21]*).

FAQ3: What are the minimum cell dose criteria for an "adequate" single unit graft?

The minimum TNC and CD34+ cell dose thresholds for single unit grafts vary between countries and are influenced by additional factors such as HLA-mismatch and malignant or non-malignant CBT indications.

Minimum TNC dose:

- The U.S. use a minimum TNC dose of > 2.5 x 10^7/kg[9, 10, 22] based on studies showing improved engraftment, transplant-related mortality and survival above this threshold[23-25].
- The U.K. and Europe have adopted a minimum dose of > 3.0 x 10^7/kg for single-unit grafts[7, 8, 26] given two registry studies demonstrated a TNC dose greater than this higher threshold was associated with reduced mortality[27, 28].
- Japan has adopted a lowest TNC dose threshold of 2.0 x 10^7/kg[12, 29] to extend access to

single unit transplants.

- TNC doses significantly greater than the accepted minimum TNC thresholds of 2.0-3.0 x 10^7/kg have been associated with improved engraftment and potentially lower mortality, especially in the presence of high degrees of HLA-disparity[5, 24, 29-31].
- Higher minimum TNC thresholds (e.g. TNC $\geq$ 4.0-5.0 x 10^7/kg) are recommended for CBT for non-malignant diseases[7, 8, 32-37].

Minimum CD34+ cell dose:

The CD34+ cell dose is the most critical determinant of hematopoietic recovery[5, 12, 13, 15, 19, 38-40]. However, an association with survival outcomes has been shown in some[13, 39, 40] but not all single unit CBT series[5, 12, 15, 19]. At this time, the minimum acceptable CD34+ cell dose threshold is not fully established. Existing U.S.[9, 10] and updated Eurocord guidelines[11] accept a minimum acceptable CD34+ cell dose of 1.5 x 10^5/kg for single unit grafts. However, a higher CD34+ cell dose is now recommended to mitigate prolonged post-transplant cytopenia (**Table 1**).

FAQ4: When is a double unit graft indicated and how should it be selected?

Patients who lack a suitable single unit can be considered for a double unit graft[41, 42]. It is well established that two units, each considered inadequate as single unit grafts, can be successfully combined in a double unit graft[41, 42]. However, two randomized studies of myeloablative CBT in children and young adults have demonstrated that adding a second unit to an adequate single unit graft is not beneficial[22, 26]. These findings suggest that dCBT should be avoided in patients who have a unit of adequate TNC dose and donor-recipient HLA-match[22, 26]. However, the two trials used different minimum TNC criteria and did not incorporate consideration of CD34+ cell dose and 8-allele HLA match. Moreover, caution is required when extrapolating these findings to adults who are more likely to receive reduced intensity conditioning and therefore may benefit from the potentially enhanced graft-versus-leukemia

effects associated with double-unit grafts[43, 44]. Use of two units also increases the chance of at least one unit with optimal engraftment potential being infused.

FAQ5: What are the minimum TNC and CD34+ cell doses for a double unit CB graft?

Both TNC and CD34+ cell doses are important in dCBT[16, 45-50]. Historically, a TNC dose ≥ 1.5×10^7/kg and a CD34+ cell dose ≥ 1×10^5/kg for each unit in a dCB graft have been the adopted minimum thresholds so as to extend transplant access to the majority of patients[7-9, 11]. However, a higher CD34+ cell dose for each unit is now recommended (**Table 1**). In dCBT, while one unit will typically provide long-term hematopoiesis, the dominant unit cannot be reliably predicted at the time of selection[49]. Therefore, the characteristics of both units are equally important and identical selection criteria should be applied to each unit. There is no data to support the consideration of the combined unit cell dose in double unit graft selection.

FAQ6: How should donor-recipient HLA-match be evaluated?

Donor-recipient HLA-match of CB units should be evaluated at 6 HLA-loci (HLA-A, -B antigen, -DRB1 allele level typing) and 8 HLA-loci (HLA-A, -B, -C, -DRB1 allele level resolution).

FAQ7: What is the minimum required donor-recipient HLA-match?

Historically, unit-recipient HLA matching has been based on HLA-A, -B antigen, -DRB1 allele-level typing (6-loci HLA-match grade)[9, 51], with the exception of Japan that accepts antigen-level HLA typing for all 6-loci[12]. However, a minimum of 8-loci HLA-A, -B, -C, -DRB1 allele-level typing (8-allele HLA-match grade) is now required in Europe[11], the U.K.[7] and the U.S.[9, 10].

HLA -A, -B antigen, -DRB1 allele HLA-match (6-loci HLA-match grade)

A minimum requirement of donor-recipient 4/6-loci HLA-match has been widely accepted[7-11, 22, 23, 26, 51, 52]. In CBT for hematologic malignancies, HLA-mismatch has been

associated with inferior engraftment, increased risk of GVHD and potentially TRM[4, 12, 13, 23, 30, 52, 53], but also lower relapse risk[4, 12, 52, 53]. Consequently, a higher degree of HLA disparity at 6-loci has been associated with inferior survival in some sCBT studies[13, 23, 30, 53], but not in others[4, 5, 12, 22, 25, 52, 54]. One study has suggested that the deleterious effect of HLA-mismatch on survival is limited to children[29].

HLA-A, -B, -C, -DRB1 allele HLA-match (8-allele HLA-match grade)

In sCBT for malignant diseases, a higher degree of 8-allele HLA-mismatch has been associated with inferior engraftment, higher rates of aGVHD and TRM, but also a lower incidence of relapse[5, 27, 55]. Inferior survival has been observed only with < 4/8 HLA-matched grafts[5, 27], or <5/8 HLA-matched grafts in children[5]. Consequently, avoidance of units that are <4/8 HLA-matched is generally recommended[9, 10], if possible.

HLA-match in double unit CBT

Presently, the recommendations for the minimum 6-loci HLA-match of each unit of a dCB graft are the same as for single units. Several studies have shown either no detrimental effect, or even benefit, of higher degrees of HLA-allele mismatch on survival post-dCBT[48, 56-58]. Consequently, a minimum donor-recipient 8-allele HLA-match requirement is not well established in dCBT. The unit-unit HLA-match does not need to be considered in dCBT[59].

Non-malignant diseases

Prioritization of well-matched units at the HLA-allele level is recommended as it has been associated with improved outcomes in CBT for non-malignant disease[6, 60, 61].

Other HLA-match considerations

Finally, for all populations, there is insufficient or conflicting data regarding CBT outcomes according to locus-specific HLA mismatches[12, 27, 28, 62-64], direction of mismatch[65-68], or 10 or 12 HLA-allele level matching[64, 69]. It is also not practical to consider non-inherited maternal antigen (NIMA) or inherited paternal antigen (IPA) matching in most patients[70-73].

FAQ8: How should unit quality be evaluated?

Unit quality is determined by banking practices and will be influenced by processing and cryopreservation techniques. The goal is to select units of high quality to maximize post-thaw cell dose recovery and potency and, thereby, the engraftment potential. The following characteristics must be considered:

Bank accreditation and licensure

Standardization of banking practices is crucial to ensure consistent product quality and reliability of testing results such as the correlation between pre- and post-thaw viable CD34+ content[16]. Accordingly, banks with FACT accreditation are preferred[9, 10]. In the U.S., FDA licensure is associated with high quality. FDA regulations ensure safety, quality, identity, potency and product purity and provide assurance that all steps from collection to unit release undergo rigorous monitoring and results meet pre-determined standards. Non-licensed units banked under similar conditions are also acceptable[6].

Cryopreservation volume

Most automated processing systems have a pre-defined, standardized final volume (approximately 25 mL with DMSO, or 50 mL in two 25 mL bags). In contrast, the volumes of manually processed units vary. Units with non-standard cryovolumes have been associated with lower post-thaw viability and, consequently, inferior engraftment potential[16, 74].

RBC content

RBC-replete units are no longer recommended given the increased likelihood of serious infusion reactions[6, 75]. Additionally, washing these units can lead to significant cell loss given the lack of a clear interface after centrifugation. RBC-replete units usually have larger cryopreservation volumes. RBC-depleted units with standard cryovolumes that result from automated processing are preferred.

Year of collection

It is well documented that CB potency and engraftment potential is preserved after many years of cryopreservation[16, 76-78]. However, most centers consider unit age in selection as banking practices have improved over time and recent units (i.e. those collected in last 10-15 years) are more likely to have undergone more optimized procedures and testing compared to those collected in earlier years.

Post-thaw segment potency

Evaluation is not widely standardized. NetCord-Fact specifications require a minimum thawed segment CD34+ cell viability ≥70%. However, transplantation of units with a higher minimum segment CD34+ cell viability of ≥80% by flow cytometry is preferred, and units with lower viability should potentially be avoided.

FAQ9: What are other measures of unit quality?

- Unit identity should be verified by HLA confirmatory typing (or a similar DNA-based assay) of an attached segment.
- Donor eligibility is based on maternal risk factors and maternal Infectious Disease Markers screening. Units from ineligible donors can be used based on FDA requirements of "Urgent Medical Need" after evaluating the potential risk associated with the reason for ineligibility versus the potential benefit of CBT with these unit(s), relative to other units or options for therapy.

FAQ10: Are units targeted by donor-specific HLA antibodies contraindicated?

The impact of donor-specific HLA-antibodies (DSA) on engraftment after CBT for hematologic malignancies is controversial but points to consider include:

- Some[8, 79-83] but not all[84, 85] studies suggest presence of DSA increase the risk for graft failure.
- DSA number, titer, locus specificity and complement binding capacity of the DSA, as well as

the graft cell dose, must be considered on a case by case basis[86, 87].

- Additional important factors include recipient diagnosis, patient's prior immunosuppressive therapy, and planned conditioning intensity because they will also influence the potential for graft rejection.
- Consideration of DSA should not significantly compromise the cell dose of the selected graft.
- Antibody debulking strategies are not standardized and cannot be relied upon to guarantee engraftment.

In CBT for non-malignant diagnoses, DSA-targeted units should be avoided.

FAQ11: What factors do not need to be taken into consideration in unit selection?

ABO mismatch has not been established as a determinant of inferior survival in CBT[88-91]. Also, as the importance of KIR typing in CBT remains inconclusive, it should not be included in unit selection at this time[92-97]. Other unit characteristics that do not require consideration are nucleated RBC content, and donor gender or ancestry.

FAQ12: What are the practical steps in CB unit selection?

A suggested step-by-step guide to the process of CB search and ultimate graft selection is shown in **Table 1**. Selection steps may be further modified by transplant centers according to expertise and center specific needs.

FAQ13: Should cell dose or HLA-match take priority in unit selection?

How to prioritize cell dose versus HLA-match is unknown. While analyses have evaluated the relative importance of TNC dose and 4-6/6 HLA-match[29, 30, 53], information as to the relative importance of CD34+ cell dose versus 8-allele HLA-match is limited[5]. Moreover, it is

important to make a distinction between the minimal acceptable cell dose or HLA-match versus what is considered optimal.

In patients (such as many children and some adults with common haplotypes) who have units with high doses (e.g. TNC $\geq$ 3 x 10^7/kg and CD34+ $\geq$ 2 x 10^5/kg), HLA-match can be prioritized. Conversely, for most adults and some larger children, cell dose may need to take priority over HLA-match and double unit grafts may be needed. In patients with difficult searches, achieving an adequately dosed graft may mandate the transplantation of units with a high degree of HLA-mismatch. Avoidance of very well matched units (i.e. 8/8 HLA-allele matched) in patients with hematologic malignancies may also be considered due to the increased risk of relapse[5, 55]. In contrast, in patients with non-malignant diseases, optimization of HLA-match is very important[60].

Overall, expert centers agree that cell dose thresholds that are higher than the minimum should be considered to minimize the risk of graft failure and avoid protracted post-transplant cytopenia (**Table 1**). Also, many centers will restrict selection to units with a donor-recipient HLA-match of at least 4/8.

FAQ14: What are important future considerations in CB unit selection?

There are many unanswered questions in CB unit selection. Two of the most common are how to prioritize cell dose versus HLA-match and the criteria for choosing single versus double unit grafts. Whether CB expansion will permit the safe transplantation of lower cell dose but better HLA-matched units is also unknown.

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Table 1. Step-by-step CB unit selection guide in the U.S.

STEP*	ACTION	COMMENTS
1	Enter patient's high-resolution HLA-typing and weight (kg) and sort units in MatchSource®.	<u>2 options for initial unit sorting</u>: - Sort by CD34+ cell or TNC dose (better matched units may appear lower on list). - Sort by HLA-match (lower dose units may appear higher on list). <u>*Note*</u>: If sorted by 8-allele HLA-match, units in MatchSource® will be listed based on the highest possible HLA-match grade.

2	Filter *out* units with low TNC dose.	Minimum TNC dose: Single unit grafts: 2.5 x 10^7/kg. Double unit grafts: 1.5 x 10^7/kg for each unit. *Note*: Higher minimum TNC cell doses are recommended (see Step 9).
3	Filter *out* units with low CD34+ cell dose.**	Minimum CD34+ cell dose: Single unit grafts: 1.5 x 10^5/kg. Double unit grafts: 1.0 x 10^5/kg for each unit. *Note*: Higher minimum CD34+ cell doses are recommended (see Step 9).
4	Filter *out* units that are highly HLA-mismatched.	Minimum 6-loci (HLA-A, -B antigen, -DRB1 allele) match: 4/6. Minimum 8-allele (HLA-A, -B, -C, -DRB1) HLA-match: 4/8.
5	Filter *out* old units	Units collected 15 years or more ago. *Note*: Older units may be considered.
6	Filter *out* non-standard cryopreservation volumes and/ or RBC replete units.	Optimal volume: 24-28 ml (1 bag) or 48-54 ml (2 bags each of 24-28 ml/bag). *Notes:* - If unit volume ≥30 mls, verify it is RBC-depleted (filter out if RBC-replete). - Rarely unit volumes are listed without including the ~5 mL of DMSO (19-21 mL). If so, verify the correct cryovolume.
7	Filter *out* units from non-FACT accredited Banks	Prioritize banks with FACT accreditation to optimize unit quality. *Note:* Avoidance of certain banks may also be considered (e.g. banks unknown to the transplant center).
8	Sort units *If the search is difficult, above filters can be relaxed or alternative stem cell sources can be considered.*	Two options for unit presentation: I. Sort by CD34+ cell dose (highest to lowest). *or* II. Sort by 8-allele HLA-match grade (if unit typed or by Haplogic® predictions): 1) List 8/8 HLA-matched units (highest to lowest CD34+ cell dose). 2) Repeat for 7/8, 6/8, 5/8, 4/8 units (within each match grade sort by dose).
9	Review and select units for confirmatory typing. *Units already typed at high resolution can be placed on hold.* *Will need 1-2 units for the graft and 1-2 domestic units as backups.*	Must consider cell dose, HLA-match and unit quality. 1) Select 4 to 6 (if possible) units with adequate TNC and CD34+ cell dose/kg and acceptable HLA-match. 2) Assess specificities and MFI of DSA (if present). *Notes*: - Minimum cell dose thresholds capture all potentially acceptable units. - Selection of units with higher cell doses is now recommended, i.e.: Single units: TNC cell dose ≥ 3.0 x 10^7/kg and CD34+ cell dose ≥ 2.0 x 10^5/kg. Double units: CD34+ cell dose ≥ 1.5 x 10^5/kg for each unit. - If CD34+/ TNC content ratio is unexpectedly high (≥1.5-2%), the listed CD34+ cell dose should be verified. - How to trade off dose versus HLA-match is not well established. If all units have a low cell dose, selection of highly HLA-mismatched units may be necessary to achieve acceptable dose. HLA-match can be optimized if multiple high cell dose units are available. - For patients with hematologic malignancies, units that are very well HLA-matched (i.e. 8/8 HLA-allele matched) may be avoided to reduce the risk of relapse. - For patients with non-malignant diseases, both cell dose and HLA-match

		need to be optimized. - Units targeted by high DSA titers should be avoided if possible. - Additional center-specific criteria may be applied in final CB unit selection.

** Steps 1-5 need to be performed in MatchSource® as of June 2020. Units of interest should then be exported into an excel file for further sorting and final unit selection.*
*** Units with adequate CD34+ cell dose which do not meet minimum TNC dose criteria may be considered if the CD34+/TNC ratio is within an acceptable range. Bank accreditation, processing, and year of cryopreservation must be considered for such units.*