REGULAR ARTICLE

blood advances

$CD34^+$ cell content of 126 341 cord blood units in the US inventory: implications for transplantation and banking

Juliet N. Barker,[1] Jane Kempenich,[2] Joanne Kurtzberg,[3] Claudio G. Brunstein,[4] Colleen Delaney,[5,6] Filippo Milano,[5,6] Ioannis Politikos,[1] Elizabeth J. Shpall,[7] Andromachi Scaradavou,[8] and Jason Dehn[2]

[1]Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY; [2]National Marrow Donor Program, Minneapolis, MN; [3]Department of Pediatrics, Duke University Medical Center, Durham, NC; [4]Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota, Minneapolis, MN; [5]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA; [6]Department of Medicine, University of Washington, Seattle, WA; [7]Department of Stem Cell Transplant and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, TX; and [8]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY

Key Points

- Because CB units with the highest TNC dose may not have an adequate $CD34^+$ dose, both should be considered in selecting CB units.
- The national US inventory of adequately sized single CB units for larger patients (>70 kg) is small.

$CD34^+$ cell dose is critical for cord blood (CB) engraftment. However, the $CD34^+$ content of the CB inventory in the United States is unknown. We examined the $CD34^+$ cell content of 126 341 red blood cell–depleted US units banked from January 2007 to September 2017 with a total nucleated cell (TNC) count of $\geq 90 \times 10^7$ and a cryovolume of 24-55 mL. Median pre-cryopreservation TNC content was 127×10^7 (interquartile range [IQR], 108-156 $\times 10^7$); $CD34^+$ cell content was 44×10^5 (IQR, 29 to 67 $\times 10^5$). The median $CD34^+$:TNC ratio was 0.34%. TNC and $CD34^+$ cell content correlation was weak ($r = 0.24$). Of 7125 units with TNCs of $\geq 210 \times 10^7$, only 47% had $CD34^+$ content of $\geq 100 \times 10^5$. However, some units had high $CD34^+$ content for a given TNC count. Only 4% of CB units were acceptable as single-unit grafts (TNCs, $\geq 2.5 \times 10^7$/kg; $CD34^+$ cells, $\geq 1.5 \times 10^5$/kg) for 70-kg patients; 22% of units were adequate for 70-kg patients using lower dose criteria (TNCs, $\geq 1.5 \times 10^7$/kg; $CD34^+$ cells, $\geq 1.0 \times 10^5$/kg) suitable for a double-unit graft. These findings highlight that units with the highest TNC dose may not have the highest $CD34^+$ dose, units with unexpectedly high $CD34^+$ content (a ratio of >1.0%) should be verified, and the US CB inventory of adequately sized single units for larger patients is small. They also support the ongoing use of double-unit grafts, a focus on banking high-dose units, and development of expansion technologies.

Introduction

Infused $CD34^+$ cell dose is a critical determinant of the speed and success of engraftment after both single-[1,2] and double-unit[3] cord blood (CB) transplants. Although lack of standardization of $CD34^+$ assays may result in measurements of $CD34^+$ content being less reliable than those for total nucleated cells (TNCs), studies of postthaw $CD34^+$ cell recovery have shown that viable $CD34^+$ content can usually be predicted at the time of unit selection in more recently collected quality units that have undergone optimized processing and cryopreservation.[3-5] Moreover, precryopreserved $CD34^+$ cell dose has been associated with engraftment success and has a greater influence on hematopoietic recovery than TNC dose.[5,6] Consequently, incorporation of the $CD34^+$ cell dose, in addition to the TNC dose, into CB unit selection is now recommended.[7,8] However, the $CD34^+$ content of the current unrelated donor National Marrow Donor Program (NMDP) Be the Match CB inventory has not been analyzed.

Methods

We examined the $CD34^+$ cell content of CB units originating from US CB banks listed in the NMDP Be the Match registry. Inclusion for analysis was limited to units banked between January 2007 and

Submitted 26 November 2018; accepted 28 February 2019. DOI 10.1182/bloodadvances.2018029157.

To request original data, contact Juliet N. Barker (barkerj@mskcc.org).

September 2017 that were red blood cell (RBC) depleted, had a TNC content of $>90 \times 10^7$, a cryovolume of 24-55 mL (1 or 2 bags), and were either available or reserved; their $CD34^+$ cell content postprocessing was reported. The cryovolume restriction of <55 mL was included because these units will have undergone optimized processing or cryopreservation, which results in RBC depletion. Most such units will have cryovolumes of approximately 25 mL (or 25 mL × 2 = ~50 mL). TNC and $CD34^+$ data were obtained from the existing NMDP Database. Excluded from this analysis were 2046 units with cryovolume <24 mL or not reported, 4054 units with frozen volume ≥56 mL, and 910 units that did not have $CD34^+$ content reported.

Published NMDP and American Society of Blood and Marrow Transplantation CB Special Interest Group definitions of adequate cell doses were used to examine the number of units in the inventory that were considered adequate for single-unit (TNCs, $\geq 2.5 \times 10^7$/kg; $CD34^+$ cells, $\geq 1.5 \times 10^5$/kg) and double-unit (TNCs, $\geq 1.5 \times 10^7$/kg/unit; $CD34^+$ cells, $\geq 1.0 \times 10^5$/kg/unit) CB grafts.[7] Descriptive statistics were used to describe the cell dose characteristics of the CB inventory, including calculations of $CD34^+$:TNC ratio and cell doses by patient weight.

Results

In all, 126 341 units collected by 20 public banks fulfilled the criteria for analysis. Of the 126 341 analyzed units, the median pre-cryopreservation TNC content was 127×10^7 (interquartile range [IQR], 108 to 156×10^7); 89 126 units (71%) had a TNC content of $<150 \times 10^7$, 20 618 units (16%) had a TNC content of 150 to 179×10^7, 9472 units (7%) had a TNC content of 180 to 209×10^7, and 7125 units (6%) had a TNC content of $\geq 210 \times 10^7$ (Table 1).

The median $CD34^+$ cell content was 44×10^5 (IQR, 29 to 67×10^5). In all, 72 665 units (58%) had a $CD34^+$ cell content of $<50 \times 10^5$, 41 844 units (33%) had a $CD34^+$ cell content of 50 to 99×10^5, 10 820 units (9%) had a $CD34^+$ cell content of 100 to 200×10^5, and only 1012 units (<1%) had a $CD34^+$ cell content of $>200 \times 10^5$ (Table 1).

Because both TNC and $CD34^+$ cell doses should be considered in unit selection,[7,8] we examined the relationship between unit TNCs vs $CD34^+$ content (Table 1). The correlation between the TNC and $CD34^+$ cell content of the CB units was weak ($r = 0.24$). Thus, in a patient's search there could potentially be many units with a high TNC count but with intermediate or low $CD34^+$ cell content. For example, of the CB units with TNC content $\geq 210 \times 10^7$ (n = 7125 units; shown in bold in Table 1), less than half (n = 3332; 47%) had $CD34^+$ cell content $\geq 100 \times 10^5$. Conversely, of units with low TNC content of $<210 \times 10^7$ (n = 119 216), a small fraction (n = 8500; 7%) had high $CD34^+$ content, defined as $\geq 100 \times 10^5$.

To evaluate the fraction of the TNC content that can be expected to be $CD34^+$ cells, we analyzed the $CD34^+$ cell:TNC content ratio of the units in the inventory (Figure 1). The median ratio was 0.34% (IQR, 0.23%-0.48%). The majority of units (n = 76 719; 61%) had a ratio between 0.2% and <0.5%. Only 20 263 (16%) had a ratio of <0.2%, whereas only 27 026 (21%) had a ratio of 0.5% to <1.0%. A ratio of ≥1.0% was very uncommon (n = 2333 units; 2%). Reported ratios did not vary significantly between banks.

We next examined the number of units that fulfill minimum cell dose criteria for a single-unit graft (TNCs, $\geq 2.5 \times 10^7$/kg; $CD34^+$ cells, $\geq 1.5 \times 10^5$/kg per current guidelines[7,9]) by patient weight. Although approximately half the inventory (n = 62 282; 49%) units met minimum criteria for a 30-kg patient and 37 788 (30%) of the units met minimum criteria for a 40-kg patient, the number of acceptable units for adult-size patients was small. For example, only 5418 units (4% of the inventory) had TNC and $CD34^+$ cell doses adequate for a patient weighing 70 kg (Figure 2, smaller panel). The number of acceptable units all US CB units for larger patients progressively diminished: 2826 (2%) for 80-kg, 1495 (1%) for 90-kg, and 776 (<1%) for 100-kg patients.

Reducing the cell dose criteria to the minimum acceptable for each unit of a double-unit graft (TNCs, $\geq 1.5 \times 10^7$/kg; $CD34^+$ cells, $\geq 1.0 \times 10^5$/kg) increased adequately sized units to 93 717 (74% of the inventory) for a 30-kg patient and 72 125 (57%) units for a 40-kg patient. For a 70-kg patient, the number increased to 27 274 (22%) (Figure 2, larger panel). The number of acceptable units for larger patients was 18 494 (15%) for 80-kg, 12 400 (10%) for 90-kg, and 8363 (7%) for 100-kg patients.

Table 1. Distribution of the combined TNC and $CD34^+$ cell content of the domestic CB unit inventory (n = 126 341).

	$CD34^+$ cells × 10^5					
	<50	**50-69**	**70-99**	**100-199**	**200+**	**Total**
TNCs × 10^7						
90-119	38 727	7 682	3 481	1 106	38	51 034
120-149	22 270	8 342	5 148	2 257	75	38 092
150-179	8 339	5 241	4 310	2 586	142	20 618
180-209	2 466	2 264	2 446	2 144	152	9 472
210-239	666	773	1 058	1 378*	166*	**4,041**
240-269	142	254	438	683*	156*	**1,673**
270-299	38	88	182	369*	100*	**777**
300+	17	35	102	297*	183*	**634**
Total	72 665	24 679	17 165	10 820	1 012	126 341

The data reflect the number of units fulfilling each cell content level. Units in bold reflect those units with a TNC content $>210 \times 10^7$/kg.
*The proportion of units that also have a $CD34^+$ cell content $>100 \times 10^5$/kg.

Discussion

This is the first analysis to evaluate the $CD34^+$ content of the US CB inventory. It should be noted that our analysis did not consider units collected before 2007. However, we do not consider this a limitation because units more recently collected are associated with optimized banking practices and are much more likely to be used. Our findings do require detailed validation, however, and could be limited by changes in postprocessing prefreeze $CD34^+$ cell enumeration assays over time. They also do not account for variations in unit quality as reflected by the postthaw $CD34^+$ cell viability.[3] Nonetheless, they provide critical information that has important implications for CB transplantation, CB banking, and CB bank funding.

From a practical standpoint, these data clearly reinforce incorporating both TNC and $CD34^+$ cell dose into CB unit selection, because units with an adequate TNC dose do not necessarily have an adequate $CD34^+$ cell dose. Centers should recognize that once $CD34^+$ dose is considered, the best units may not necessarily have the highest TNC dose. Importantly, incorporation of this guidance into unit selection could improve engraftment without the need for CB expansion and therefore could have major advantages in terms of transplantation costs and use of CB.

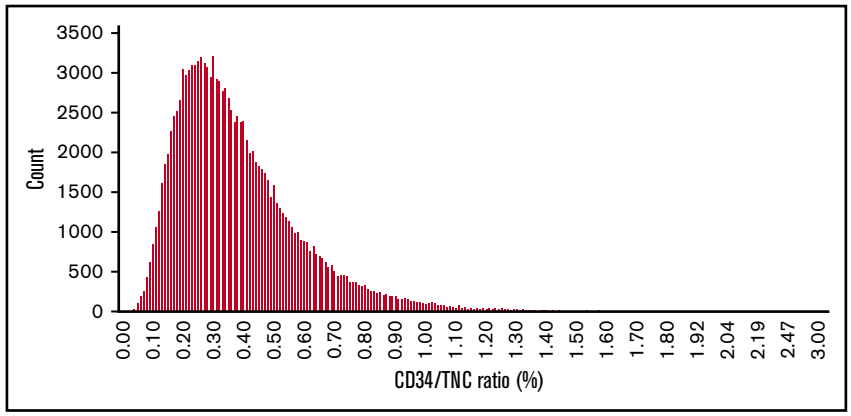

Figure 1. Distribution of the CD34:TNC ratio of CB units in the domestic CB unit inventory. A total of 23 units with ratios of 3.0 to 30.9 are excluded from this figure.

The potential clinical implications of the CD34$^+$:TNC content ratio are unknown and require investigation. It is critically important that if transplant centers incorporate CD34$^+$ dose into unit selection, the precryopreserved CD34$^+$ dose must approximate what will be recovered after thawing. Current guidance states that centers should request unit reports and CB bank verification of units with a CD34$^+$ dose much higher than expected based on both the TNC dose and the CD34$^+$:TNC ratio (eg, >1%). The implications of an unexpectedly high CD34$^+$ dose are complex. Such a unit may be very good because it is enriched with CD34$^+$ progenitors. Conversely, the reported CD34$^+$ cell content could be wrong. This would most likely result from an error in software data entry.

A valid cutoff of the expected CD34$^+$ content for a given TNC dose has not been established and requires further detailed investigation. However, the observed range of the CD34$^+$:TNC ratio in this study provides guidance on what may be an expected CD34$^+$ content relative to the reported precryopreservation TNC count. In this analysis, a ratio of ≥1.0% was very uncommon (2% of the inventory). Therefore, a CD34$^+$:TNC content ratio of 2% or 3%, for example, should be questioned. The CD34$^+$ cell content and potency of a prefreeze specimen, an associated cryovial or attached segment[10-12] could potentially be informative in this scenario, and each requires investigation. In addition, whether the CD34$^+$:TNC ratio has any clinical association with engraftment, independent of the infused doses of TNCs and viable CD34$^+$ cells, also requires investigation. Such analyses will require compiling data from multiple dedicated CB transplant centers from their cell therapy laboratories and their clinical databases. Analyses will need to include a patient population transplanted with relatively uniform myeloablation and immunosuppression, as well as examining any variations in thaw techniques and CD34$^+$ cell enumeration assays between centers.

Our analysis highlights the fact that there is a large inventory of CB units with adequate TNC and CD34$^+$ doses for patients of low to intermediate weight. This has major implications for pediatric transplantation (and smaller adults), because these units can be obtained quickly, and high survival rates have been achieved with pediatric CB transplant.[9,13] The number of single units for patients weighing at least 70 kg, however, is relatively small, which supports the continued use of double-unit CB grafts for patients without adequately sized single CB units. Such grafts have been associated with very high engraftment rates in larger patients,[3,14,15] albeit with delayed count recovery relative to transplantation of mobilized peripheral blood. Moreover, we found that lowering the cell dose threshold to the minimum considered acceptable for double-unit

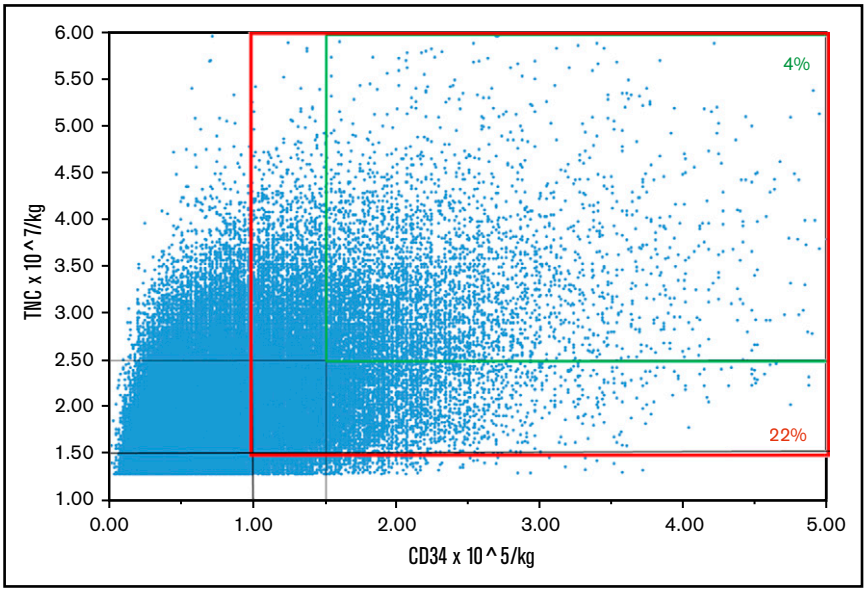

Figure 2. Potential units that are adequately dosed for a 70-kg patient. Suitable units for a single-unit graft (TNC dose, ≥2.5 × 10^7/kg; CD34$^+$ cell dose, ≥1.5 × 10^5/kg) represent 4% of the inventory (smaller panel). Units that would be suitable as 1 of 2 for a double-unit graft (TNC dose, ≥1.5 × 10^7/kg; CD34$^+$ cell dose, ≥1.0 × 10^5/kg per unit) represent 22% of the inventory (larger panel).

grafts notably increased the number of CB units that would be available for larger children and adults.

Our analysis also supports the need for concerted efforts and ongoing funding to enrich the CB inventory with units that have high cell doses. This approach has recently been the focus of many CB banks and offers the advantage of increased cost-effectiveness because CB units with higher cell content are more likely to be used. Finally, new technologies such as ex vivo CB expansion or the addition of cells from a third party could potentially further improve engraftment and are currently under investigation.[16-22] These technologies could also enlarge the usable CB inventory, which has relevance when a better HLA-matched unit is required for transplantation.

Acknowledgments

The authors thank the participating banks that provided and verified the unit data.

This work was supported in part by US Health Resources and Services Administration contracts HHSH234200637021C and HHSH250201200018C.

Authorship

Contribution: All authors designed and performed the research, analyzed the data, and helped write the paper.

Conflict-of-interest disclosure: J.N.B. received clinical trial or educational funding from NYSTEM, Angiocrine Bioscience, and Gamida Cell. J. Kempenich and J.D. are employees of the National Marrow Donor Program (NMDP). J. Kurtzberg is medical director of CryoCell Cord Blood Bank. C.D. is chief scientific officer of Nohla Therapeutics. J. Kurtzberg, A.S., and E.J.S. are medical directors of public CB banks (New York Blood Center's National Cord Blood Program, Carolinas CB Bank Duke University, and MD Anderson Cancer Center CB Bank, respectively). J.N.B., J. Kurtzberg, F.M., E.J.S., and A.S. are medical advisors to the NMDP. The remaining authors declare no competing financial interests.

Correspondence: Juliet N. Barker, Memorial Sloan Kettering Cancer Center, 1275 York Ave, Box 259, New York, NY, 10065; e-mail: barkerj@mskcc.org.

References

1. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood*. 2002;100(5):1611-1618.
2. Page KM, Zhang L, Mendizabal A, et al. Total colony-forming units are a strong, independent predictor of neutrophil and platelet engraftment after unrelated umbilical cord blood transplantation: a single-center analysis of 435 cord blood transplants. *Biol Blood Marrow Transplant*. 2011;17(9):1362-1374.
3. Purtill D, Smith K, Devlin S, et al. Dominant unit CD34+ cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice. *Blood*. 2014;124(19):2905-2912.
4. Lemarie C, Esterni B, Calmels B, et al. CD34(+) progenitors are reproducibly recovered in thawed umbilical grafts, and positively influence haematopoietic reconstitution after transplantation. *Bone Marrow Transplant*. 2007;39(8):453-460.
5. Konuma T, Kato S, Oiwa-Monna M, et al. Cryopreserved $CD34^{+}$ cell dose, but not total nucleated cell dose, influences hematopoietic recovery and extensive chronic graft-versus-host disease after single-unit cord blood transplantation in adult patients. *Biol Blood Marrow Transplant*. 2017;23(7):1142-1150.
6. Sanz J, Sanz MA, Saavedra S, et al. Cord blood transplantation from unrelated donors in adults with high-risk acute myeloid leukemia. *Biol Blood Marrow Transplant*. 2010;16(1):86-94.
7. Barker JN, Kurtzberg J, Ballen K, et al. Optimal practices in unrelated donor cord blood transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2017;23(6):882-896.
8. Hough R, Danby R, Russell N, et al. Recommendations for a standard UK approach to incorporating umbilical cord blood into clinical transplantation practice: an update on cord blood unit selection, donor selection algorithms and conditioning protocols. *Br J Haematol*. 2016;172(3):360-370.
9. Wagner JE Jr, Eapen M, Carter S, et al; Blood and Marrow Transplant Clinical Trials Network. One-unit versus two-unit cord-blood transplantation for hematologic cancers. *N Engl J Med*. 2014;371(18):1685-1694.
10. Faivre L, Boucher H, Zerbib R, et al. Cord blood attached segment: is this a relevant quality control to predict a good hematopoietic stem cell graft? *Bone Marrow Transplant*. 2017;52(9):1353-1354.
11. Lee HR, Shin S, Yoon JH, et al. Attached segment has higher CD34+ cells and CFU-GM than the main bag after thawing. *Cell Transplant*. 2015;24(2):305-310.
12. Scaradavou A, Dobrila L, Albano MS, et al. Cord blood (CB) stability and potency evaluation: consistent, predictable recovery of hematopoietic progenitor cells (HPC) and high CD34+ cell viability in stored cord blood units (CBU) of the National Cord Blood Program (NCBP) [abstract]. *Blood*. 2016;128(22). Abstract 2175.
13. Eapen M, Kurtzberg J, Zhang MJ, et al. Umbilical cord blood transplantation in children with acute leukemia: impact of conditioning on transplantation outcomes. *Biol Blood Marrow Transplant*. 2017;23(10):1714-1721.
14. Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 2005;105(3):1343-1347.
15. Scaradavou A, Brunstein CG, Eapen M, et al. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood*. 2013;121(5):752-758.

16. Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med*. 2010;16(2):232-236.

17. de Lima M, McNiece I, Robinson SN, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med*. 2012;367(24):2305-2315.

18. Butler JM, Gars EJ, James DJ, Nolan DJ, Scandura JM, Rafii S. Development of a vascular niche platform for expansion of repopulating human cord blood stem and progenitor cells. *Blood*. 2012;120(6):1344-1347.

19. Fares I, Chagraoui J, Gareau Y, et al. Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science*. 2014;345(6203):1509-1512.

20. Popat U, Mehta RS, Rezvani K, et al. Enforced fucosylation of cord blood hematopoietic cells accelerates neutrophil and platelet engraftment after transplantation. *Blood*. 2015;125(19):2885-2892.

21. Wagner JE Jr, Brunstein CG, Boitano AE, et al. Phase I/II trial of StemRegenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. *Cell Stem Cell*. 2016;18(1):144-155.

22. Horwitz ME, Wease S, Blackwell B, et al. Phase I/II study of stem-cell transplantation using a single cord blood unit expanded ex vivo with nicotinamide. *J Clin Oncol*. 2019;37(5):367-374.