Current Cord Blood Banking Concepts and Practices

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2.1 Introduction

Umbilical cord blood (CB) is firmly established as an unrelated donor source for hematopoietic cell transplantation (HCT) and has the potential to play an important role in the evolving fields of regenerative medicine and cellular therapies. Currently, there are over 160 public banks with a global inventory of over 700,000 fully characterized, high-quality cord blood units (CBUs) (http://www.bmdw.org n.d.). Family (or private) CB banks are also available for those families electing to pay a fee to store their baby's CB for their own use. Approximately four million CBUs have been banked at an estimated 215 family banks through this mechanism (Ballen et al. 2015). As indications for autologous and allogeneic CB continue to expand, the decision facing pregnant women, whether to altruistically donate CB or bank privately as a form of "medical insurance," will become more complex. In this chapter, we review the history and current state of CB banking as well as challenges confronting the banking community.

2.2 The Historical Perspective

Over 30 years ago, it was recognized that CB was a rich source of hematopoietic stem and progenitor cells (HSPCs). In a pivotal series of experiments, Dr. Ted Boyce, working with Dr. Hal Broxmeyer and colleagues, demonstrated that CB HSPCs showed high proliferative potential, which could successfully repopulate hematopoiesis in murine models, and tolerated cryopreservation and thawing with

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M. Horwitz, N. Chao (eds.), *Cord Blood Transplantations*, Advances and Controversies in Hematopoietic Transplantation and Cell Therapy,
DOI 10.1007/978-3-319-53628-6_2

efficient HSC recovery (Broxmeyer et al. 1989). This critical work provided the scientific rationale to evaluate CB as a potential donor source of HSPCs in humans. The first patient to undergo a CB transplant (CBT) was a 5-year-old boy with Fanconi anemia and whose mother was pregnant with an unaffected, fully human leukocyte antigen (HLA)-matched sibling. In preparation for the transplant, the sibling's CB was collected into a sterile bottle containing preservative-free heparin. The CB was then transported to Dr. Broxmeyer's laboratory, diluted with tissue culture media and dimethyl sulfoxide (DMSO), cryopreserved, and stored under liquid nitrogen until it was transported in a dry shipper to Paris, France. In 1988, Dr. Eliane Gluckman performed the first CBT in the world using the sibling's CB as the donor (Gluckman et al. 1989). He successfully engrafted with his sister's cells and remains healthy with full donor chimerism 28 years later. Building on this initial success, additional related donor CBTs were performed in selected centers over the next 5 years (Wagner et al. 1992, 1995; Kohli-Kumar et al. 1993; Broxmeyer et al. 1991). Supported by a pilot grant from the National Heart, Lung, and Blood Institute (NHLBI), Dr. Pablo Rubinstein established the first unrelated donor CB bank at the New York Blood Center in 1992. In the following year, Dr. Joanne Kurtzberg performed the first unrelated donor CBT at Duke University in a 4-year-old child with relapsed T-cell leukemia. The early experience with this child and 24 additional patients transplanted over the next 2 years at Duke demonstrated that partially HLA-mismatched, banked unrelated donor CB could successfully restore hematopoiesis. Engraftment was associated with the total nucleated cell (TNC) dose available relative to the recipient body size, and the incidence of GvHD was lower than expected (Kurtzberg et al. 1996). Shortly thereafter, Wagner et al. published their experience using banked, unrelated CB in 18 recipients with similar findings reported (Wagner et al. 1996). In 1996, the NHLBI funded the cord blood transplantation (COBLT) study to prospectively test the use of unrelated donor CBT in children and adults with diseases commonly treated with HCT. Through this program, three additional unrelated donor banks were established. Standard operating procedures (SOPs) were created for donor recruitment, collection, shipping, processing, testing, long-term storage, and distribution of CBUs (Fraser et al. 1998). Over 11,000 well-characterized diverse CBUs were banked to support multicenter transplantation protocols (Cairo et al. 2005; Kurtzberg et al. 2005). Over the next 5 years, CB was tested as a donor source for children with leukemia, congenital immunodeficiency syndromes, and inherited metabolic diseases and adults with leukemia (Kurtzberg et al. 2005, 2008; Martin et al. 2006; Cornetta et al. 2005; Wall et al. 2005).

With the extension into the unrelated donor setting, the fields of CBT and banking expanded rapidly. In 1995, EUROCORD was established by Dr. Eliane Gluckman and continues to operate on behalf of the European Group for Blood and Marrow Transplantation as an international registry of CBT. In 1996, the parent organizations, International Society for Cellular Therapy (ISCT) and the American Society of Blood and Marrow Transplantation (ASBMT), established the Foundation for the Accreditation of Cellular Therapy (FACT). In 1997, the International NetCord Foundation was established to serve as a registry for international public CB banks. The members of NetCord subsequently created the first international

standards for public CB banking. FACT and NetCord established a joint collaboration to produce the first international standards for accreditation for public CB banks in 1999. In the USA, the National Marrow Donor Program (NMDP) established the Center for Cord Blood in 1998, adding CBUs to their listings on the unrelated donor registry. In 2005, legislation was passed in the US Congress to establish the CW Bill Young Cell Transplantation Program. This program created coordinating centers for CB and adult donors, a single point of access donor registry (both administered through the NMDP), a stem cell outcome database (administered by the Center for International Blood and Marrow Transplant Research, CIBMTR), and the National Cord Blood Inventory (a US network of public banks, NCBI) administered through the Health Resources and Services Administration (HRSA) of the Department of Health and Human Services.

2.3 Overview of Donor Recruitment and Consent

Donor recruitment begins with the identification of potentially eligible mothers as defined by the individual bank based on maternal and infant characteristics. For example, the Carolinas Cord Blood Bank (CCBB) will accept donations from healthy mothers (≥18 years old) who are carrying a healthy term or near-term (≥34-week gestation) singleton gestation. Eligible mothers willing to donate must sign informed consent prior to collection. Some banks use a "mini consent" which grants permission for CB collection and is signed prior to active labor followed by a more extensive informed consent addressing usage of the CBU obtained after delivery and collection. Consent includes permission to collect and potentially bank the CBU for public use or utilize for research if the unit doesn't meet specifications for banking; provide a medical and family history, for the mother to provide a blood specimen to screen for certain communicable diseases; and review medical records of the infant and maternal donors.

2.4 Overview of Collection Techniques

Cord blood can be collected from either vaginal or cesarean births, either prior to delivery of the placenta (in utero) by obstetrical (OB) staff or after delivery of the placenta (ex utero) allowing for trained CB staff to perform collections. Reports have generally observed higher collection volumes after cesarean compared to vaginal deliveries (Kurtzberg et al. 2005; Jones 2003; Santos et al. 2016) and when CB is collected in utero compared to ex utero (Solves et al. 2003a), although reports have been conflicting (Lasky et al. 2002). In an analysis of collections facilitated by the CCBB ($n = 59{,}794$), cesarean deliveries yielded higher collection volume (average 14 ml higher (95% CI 13.6–14.5), $p < 0.0001$) compared to vaginal deliveries after controlling for collection method. Contrary to other reports, ex utero collections resulted in higher collection volumes (average 5 ml higher (95% CI 4.9–5.8), $p < 0.0001$) compared to in utero after adjusting for delivery method. We also

observed an interaction between delivery type and collection method. Delivery by cesarean section yielded collection volumes that were on average 15 ml higher when collected ex utero and 10.5 ml higher when collected in utero, as compared to vaginal deliveries ($p < 0.0001$). Our results also demonstrated that cesarean deliveries collected ex utero had a median volume 19 ml higher than in utero collections from vaginal deliveries. While the delivery method is dictated by the clinical status of the mother and infant, the method of collecting is determined by staffing and collection site practices. Currently, both collection methods continue to be routinely used, but in utero collections are more common, likely due to the additional personnel expenses associated with ex utero collections.

Cord blood is typically collected by cannulating the umbilical vein to allow the placental blood to be removed by gravity into collection containers with anticoagulant, most commonly citrate phosphate dextrose (CPD). While collections occurred in open systems in the early days of CB banking, closed system collection bags were shown to reduce bacterial contamination rates (Bertolini et al. 1995) and are now routinely utilized.

Utilization of publicly banked unrelated donor CBUs in patients is based on delivery of a minimal TNC/kg dose of CB cells. Banks establish thresholds for banking based on the total nucleated cell count (TNCC) and estimate whether or not a particular unit will meet this threshold at various time points during the collection and banking process. After the collection is completed, most banks measure the weight of the collection bag to estimate the collection volume. It is well established that collection volume and TNCC are closely correlated (Fig. 2.1; Table 2.1), and many banks have established minimal volume thresholds to determine which CBUs are shipped to the processing lab. Units with low volume are unlikely to have sufficient TNCC and therefore are discarded at the site. Alternatively, some banks will measure TNCC at the collection site, or use other criteria, to determine which units should be shipped to the processing laboratory.

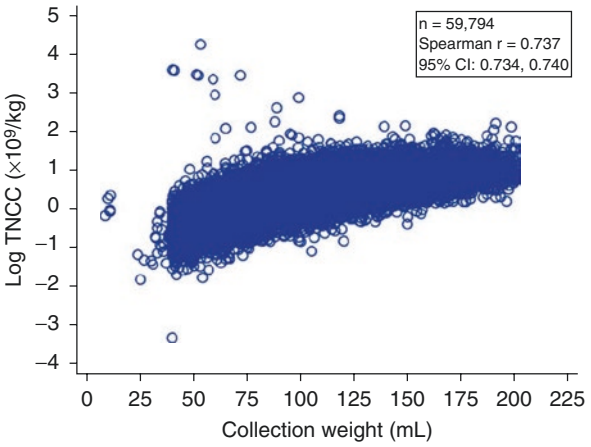

Fig. 2.1 Comparison of pre-processing TNCC and collection volume. In the scatterplot, the pre-processing TNCC (transformed logarithmically) and collection volume for cord blood units collected by the Carolinas Cord Blood Bank ($n = 59{,}794$ collections) are compared (Spearman's correlation coefficient = 0.737, $p < 0.0001$)

Table 2.1 The probability of donated cord blood units containing $<1 \times 10^9$, $1–1.75 \times 10^9$ or $>1.75 \times 10^9$ total nucleated cell content (TNCC) pre-processing based on the collection volume

	Pre-processing TNCC					
	$<1 \times 10^9$		$1 \times 10^9 – 1.75 \times 10^9$		$>1.75 \times 10^9$	
Collection weight (mL)	*N*	Proportion (95% CI)	*N*	Proportion (95% CI)	*N*	Proportion (95% CI)
<60	28,592	0.552 (0.547–0.556)	1,404	0.046 (0.043–0.048)	39	0.003 (0.002–0.004)
60–80	16,193	0.312 (0.309–0.317)	9,396	0.305 (0.300–0.311)	762	0.052 (0.048–0.056)
>80–100	4,991	0.096 (0.094–0.099)	11,420	0.371 (0.366–0.377)	3,299	0.224 (0.218–0.231)
>100–125	1,493	0.029 (0.027–0.030)	6,777	0.220 (0.216–0.225)	5,285	0.359 (0.352–0.367)
>125–150	385	0.007 (0.007–0.008)	1,494	0.049 (0.046–0.051)	3,346	0.228 (0.221–0.234)
>150	165	0.003 (0.003–0.004)	272	0.009 (0.008–0.010)	1,970	0.134 (0.129–0.140)
TOTAL	*51,819*		*30,763*		*14,701*	

TNCC total nucleated cell content, *CI* 95% Clopper-Pearson confidence interval

Efforts to increase collection volume have focused on two general approaches: identifying donations likely to have higher collection volume or developing techniques to obtain the maximal volume from an individual donation. Multiple reports have demonstrated relationships between characteristics of the mother, infant, or delivery with increased collection volume, TNCC, $CD34^+$, or colony-forming units (CFU) content of CBUs. Increased donor birth weight and older gestational age have been closely associated with higher collection volume and TNCC (Askari et al. 2005; George et al. 2006; Ballen et al. 2001), although our data which showed collections from younger infants (34–37 weeks gestation) were more likely to have higher progenitor cell content as measured by $CD34^+$ and CFU content (Fig. 2.2) (Page et al. 2014). While several studies have demonstrated comparable collection volumes among donors of races or ethnicities, the TNCC, $CD34^+$, and CFU content, all adjusted for collection volume (counts/mL), were significantly lower in African-American donors compared to Caucasian donors even after adjusting for other clinical factors (Fig. 2.3) (Kurtzberg et al. 2005; Page et al. 2014). This is likely due to differences in cellular adherence between Caucasian and African-American individuals (Reiner et al. 2011). Other clinical factors, such as gender and maternal age, have been investigated, but results have been less conclusive (Jones 2003; Page et al. 2014; Jan et al. 2008;

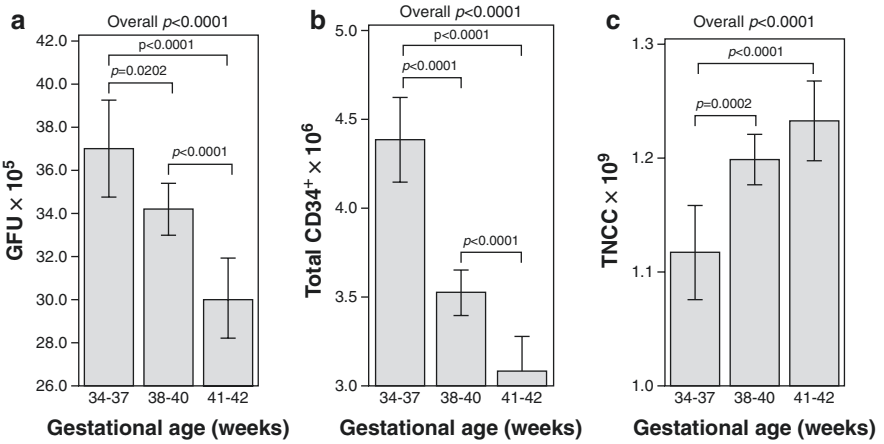

Fig. 2.2 Impact of infant-estimated gestational age on the CFU, CD34⁺, and post-TNCC content. In (**a**–**c**), the adjusted mean CFU (**a**), CD34⁺ (**b**), and post-TNCC (**c**) is shown in relationship to infant gestational age after adjusting for infant race/ethnicity, birth weight, sex, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% CIs (Used with permission) (Page et al. 2014)

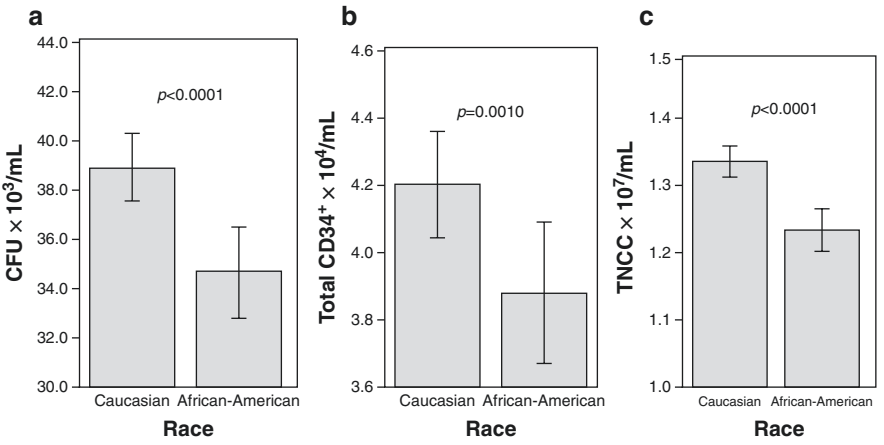

Fig. 2.3 Comparison of the CFU, CD34⁺, and post-TNCC concentrations for Caucasian and African-American infants. In (**a**–**c**), the adjusted mean CFUs/mL (**a**), CD34⁺/per mL (**b**), and post-TNCC/mL (**c**) is shown in relationship to race for infants of Caucasian and African-American race, respectively, after adjusting for infant gestational age, birth weight, sex, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% CIs (Used with permission) (Page et al. 2014)

Solves et al. 2012). Understanding these relationships between clinical characteristics and collection volume or other CB measurements, while typically not modifiable, can help to inform practical decisions such as banking eligibility, staffing at collection sites, etc.

Technical approaches to increasing CBU collection volume include increasing perfusion of the placenta to collect additional blood (Bornstein et al. 2005; Tan et al. 2009), but these approaches remain experimental. The timing of cord clamping also affects the volume of blood collected from a placenta. The practice of delayed cord clamping, defined by the American Congress of Obstetricians and Gynecologists (ACOG) as occurring >30 s after delivery (ACOG 2017), is becoming more common. While studies have shown benefits of delayed cord clamping for preterm infants, the benefits in term infants appear to be marginal (McDonald et al. 2014). Delays in collections have been associated with smaller volumes (Frändberg et al. 2016) and corresponding TNCCs and do increase collection failures due to clotting (Jones 2003; Allan et al. 2016; Solves et al. 2003b). Furthermore, there is ample evidence that the blood flow within the umbilical vessels immediately after birth is influenced by multiple physiologic factors, most notably infant lung aeration (Hooper et al. 2016). Therefore, it is not a simple time-dependent process. While it is apparent that further studies are needed to better understand the impact of cord clamping on the neonate, it is also clear that this will be an ongoing discussion with important obstetric, perinatal, and banking implications.

2.5 Overview of Current Processing and Cryopreservation Techniques

Currently, many banks receive collections from distant sites, and, therefore, delays in processing related to travel might exist. Results of the COBLT study indicated that TNCC and $CD34^+$ content remained relatively stable at room temperature for >48 h leading to the practice that cryopreservation of a processed CBU must begin within 48 h of collection (Kurtzberg et al. 2005). Others have demonstrated decreases in viability and cell content when aliquots were tested from 24–96 h after collection (Pereira-Cunha et al. 2013; Louis et al. 2012; Solomon et al. 2010; Guttridge et al. 2014). Our own experience has demonstrated small but significant losses of TNCC, $CD34^+$ cells, and CFU content at even earlier time points (Fig. 2.4) (Page et al. 2014) with similar findings reported recently by others (Wu et al. 2015; Dulugiac et al. 2014). We have therefore modified our standard operating procedures at the CCBB to prioritize processing of CBUs within 24 h of collection.

The overall approach to processing CB is similar between banks, although variations in technique do exist. Rubinstein et al., in their pivotal work, demonstrated that volume reduction achieved through plasma and red blood cell (RBC) depletion allowed for more efficient processing, cryopreservation, and cell recovery after thaw (Rubinstein et al. 1995). To this day, most CB banks employ these processing methods, or variations of it, to achieve plasma and RBC depletion. While manual CB processing continues to be performed in some banks, an increasing number of banks are using automated systems for plasma and RBC reduction. A comparison between two automated systems, Sepax© (Biosafe, Switzerland) and AutoXpress Platform or AXP© (Cesca Therapeutics, Rancho Cordova, CA), was performed at the Valencia CB bank. Both systems demonstrated acceptable cell recovery. The

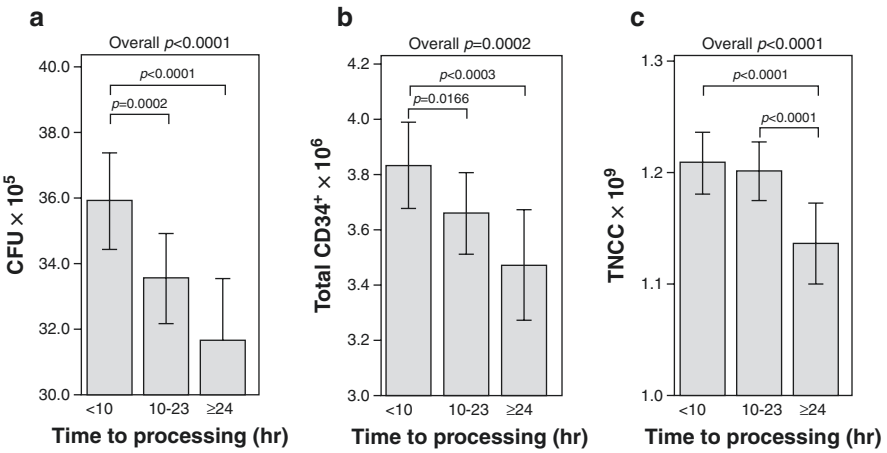

Fig. 2.4 Impact of time to processing on CFU, CD34⁺, and post-processing TNCC content. In (**a**–**c**), the adjusted mean CFUs (**a**), CD34⁺ (**b**), and post-processing TNCC (**c**) by time to processing is presented after adjusting for infant race/ethnicity, sex, gestational age, birth weight, collection volume, delivery type, and maternal age. Only significant *p* values are shown. Whisker plots represent the 95% confidence intervals (Used with permission) (Page et al. 2014)

Sepax© system, using hydroxyethyl starch (HES) as a sedimentation agent, did have improved TNCC recovery, whereas the AXP© system was especially efficient in RBC removal without HES. Recent issues with HES availability in Europe led Schwandt et al. to develop and validate a non-HES Sepax© protocol (Schwandt et al. 2016). Comparable post-processing recoveries were achieved although lower post-thaw CD34⁺ viability was noted with the non-HES protocol supporting the recommendation that HES protocols are preferred. Other automated systems for CB processing include PrepaCyte-CB (BioE, St. Paul, MN) and Macopress Smart (Macopharma, Mouvaux, Fr). Post-processing, DMSO, typically in a final concentrations of 10% along with 5% dextran or HES, is added as a cryoprotectant (Fry et al. 2013; Lecchi et al. 2016). Other concentrations of DMSO and other agents (i.e., trehalose) have been investigated, but DMSO (10%) in dextran continues to be the most commonly used cryoprotectant (Motta et al. 2014). Cryopreservation occurs via controlled-rate freezing before storage in the liquid or vapor phase of liquid nitrogen for long-term storage at less than −180 °C.

2.6 Cord Blood Banking Standards and Regulations

To ensure quality, CB products are available for patient use; standards have been developed by accrediting agencies, e.g., FACT/NetCord and AABB (formerly the American Association of Blood Banks), for CB collection, processing, and banking. These standards are the result of evidence-based consensus and establish minimal acceptable practices. Although participation is considered voluntary, many public CB banks are required to receive accreditation from FACT/NetCord or AABB to

participate in registries, receive reimbursements, etc. Many countries now regulate CB products in an effort to ensure quality and safety. In the USA, the Food and Drug Administration (FDA) regulates unrelated donor CB as biological product and issued final guidance for public banks to obtain a Biological License Agreement (BLA) in 2011. Currently six public banks in the USA have obtained a BLA.

2.7 Assessing Quality and Potency of a CBU

Banking standards require that CB products be extensively tested and characterized to assess purity, potency, and sterility of the CB unit. Testing in most banks includes assessing post-processing viability, TNCC, viable $CD34^+$ cells, growth of CFUs, and sterility. In the sections below, we discuss different methods of assessing quality and potency, review benefits and disadvantages to the assays, and briefly review the clinical impact of these measures.

2.7.1 Viability

Assessing viability is included in the banking standards for accreditation and is required for unit licensure. Guidelines require at least 85% viable cells as measured on post-processed samples. While fresh CB generally has high viability, insults to cells that can decrease viability include temperature excursions, longer time to processing, and prolonged exposure to DMSO prior to cryopreservation (Solomon et al. 2010; Dulugiac et al. 2014; Fry et al. 2013). The various cell populations contained in CB tolerate these stressors differently (Solomon et al. 2010). For example, decreases in viability may simply reflect cell death of mature granulocytes and may not reflect loss of HSPCs.

Historically, viability has been measured by staining for dying cells with trypan blue (TB) and scored either manually or using automated systems. TB is difficult to standardize and is generally felt to overestimate cell viability. More sensitive methods have been developed in the past decade and include acridine orange (AO), propidium iodide (PI), and 7-amino-actinomycin D (7-AAD) or annexin V. AO and PI are nucleic acid-binding dyes used commonly to measure cell viability. AO can pass freely into nucleated cells generating a green fluorescence. Whereas PI enters cells with compromised membranes, the red fluorescence emitted indicates a dying or necrotic cell. Automated systems allow for images to be captured and viability to be calculated. While the TB and AO/PI assays are rapid and technically easy to perform, both assays may overestimate the viability of samples by measuring only necrotic cells that have lost cell surface integrity. Nonviable cells that are earlier in the apoptotic pathway will not score positive. Other methods of assessing viability include measurement of 7-AAD or annexin V by flow cytometry. 7-AAD is a fluorescent DNA dye, whereas annexin V binds to the extracellular phosphatidylserine of early apoptotic cells. Both are able to distinguish cells earlier in the apoptotic pathway allowing for more accurate assessments of viability (Solomon et al. 2010;

Radke et al. 2013; Duggleby et al. 2012). Flow cytometry-based viability assays also allow for the viability of specific subsets to be assessed, which will be discussed further below.

2.7.2 CD34⁺ Cell Content

As a surface marker of HSPCs, it is a common practice to enumerate viable CD34⁺ cells prior to cryopreservation and again after thawing for transplantation. Efforts to standardize CD34⁺ measurements led to the development of guidelines by ISHAGE (International Society for Hematotherapy and Graft Engineering) (Sutherland et al. 1996). This "dual platform" method determined the percentage of CD34⁺ cells by flow cytometry and measured the leukocyte count using an automated cell counter. Subsequently, "single platform" approaches have been developed that enumerate CD34⁺ cells using flow cytometry (Brocklebank and Sparrow 2001; Sutherland et al. 2009). Most recently, FDA-cleared kits to enumerate viable CD34 cells have become available and adopted for use by many CB banks.

The importance of CD34⁺ cell dosing in CB grafts was demonstrated early on by Wagner et al. in 102 patients with malignant or nonmalignant diseases who received a single-unit CBT. Patients who received $>1.7 \times 10^5$/kg CD34⁺ cells infused experience higher rates of engraftment, less transplant-related mortality, and improved overall (Wagner et al. 2002). As such, some transplant centers utilize the total CD34⁺ cell dose in CBU selection recognizing that significant interlaboratory variability exists (Lemarie et al. 2007; Dzik et al. 1999; Moroff et al. 2006; Wagner et al. 2006). In our series of 435 recipients of CBT, we demonstrated that the post-thaw total CD34⁺ dose measure using the ProCOUNT© assay (BD Biosciences, San Jose, CA) was a significant predictor of neutrophil engraftment in multivariate analysis ($p = 0.04$) but to a lesser degree than post-thaw CFU ($p < 0.0001$). The total CD34⁺ dose was also weakly associated with overall survival at 6 months posttransplantation (Fig. 2.5) (Page et al. 2011a).

The presence of total CD34⁺ cells in a given CBU does not assess the viability and overall potency of the unit. This led to interest in measuring the viable CD34⁺ content. Previously, banks indirectly assessed the viable CD34⁺ content using the percent viable cells to adjust the total CD34⁺ dose. More recently, multiparametric flow cytometry methods have been developed to measure CD34⁺ in the presence of a viability marker (7-AAD; Stem Cell Enumeration Kit, BD Biosciences, San Jose, CA; Stem-kit, Beckman Coulter, Brea, CA) (Sutherland et al. 2009; Preti et al. 2014). While validation studies have demonstrated that the total and viable CD34⁺ content in fresh cord correlate closely (Preti et al. 2014; Dauber et al. 2011; Massin et al. 2015), measurements performed on thawed CB samples show more variability (Dauber et al. 2011). To date, it remains unclear how the viable CD34⁺ content of a CB graft will impact clinical outcomes since very little data is available in the literature. Purtill et al. investigated the impact of the viability of CD34⁺ cells measured post-thaw in adult patients receiving double CBT. A higher viable CD34⁺ cell dose correlated with faster engraftment kinetics in the

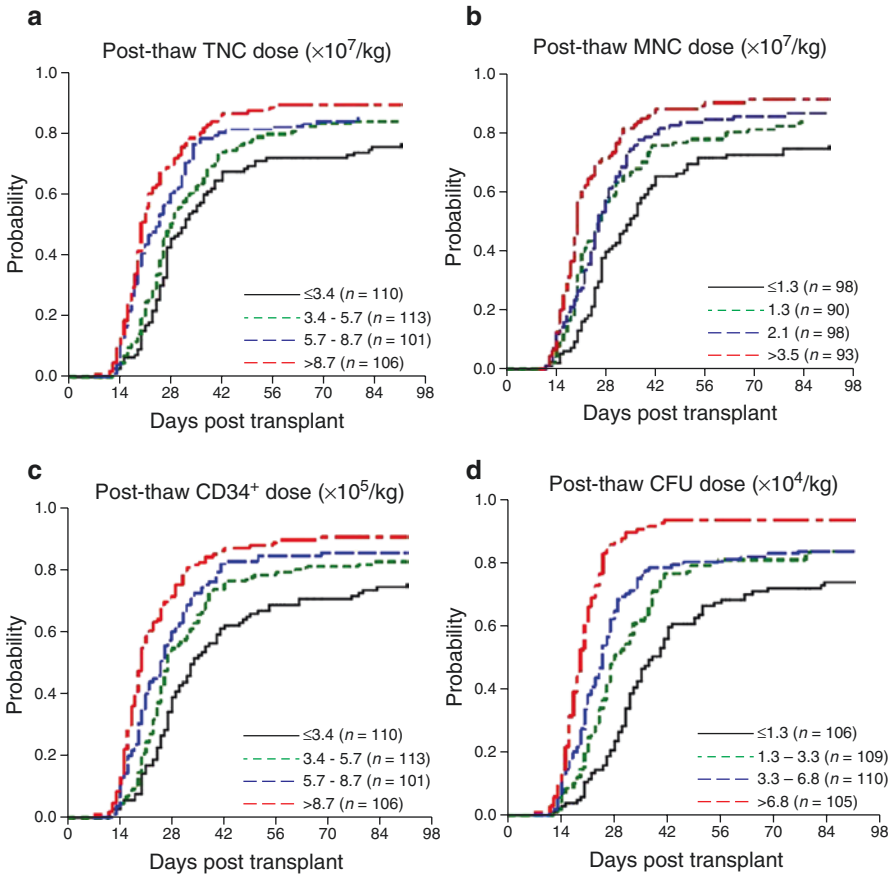

Fig. 2.5 Impact of post-thaw graft characteristics on the probability of neutrophil engraftment. Probability plates are shown for each of the four quartiles. Panels (**a**–**d**) depict the impact of post-thaw TNC ($\times 10^7$/kg recipient weight), MNC ($\times 10^7$/kg recipient weight), $CD34^+$ ($\times 10^5$/kg recipient weight), and CFU ($\times 10^4$/kg recipient weight) doses, respectively, on neutrophil engraftment (Used with permission) (Page et al. 2011a)

engrafting unit (Purtill et al. 2013, 2014). The use of the viable CD34 in CB unit selection will require further standardization of the methods for CD34 enumeration by the CB banking community.

2.7.3 Colony-Forming Units (CFUs)

CFU growth is used by many banks as a measure of potency and can be tested on a sample of processed CB or on a thawed segment post-cryopreservation. Identification and enumeration of colony types (CFU-GM, CFU-GEMM, and BFU-E) are performed by some banks, but specifications for these parameters are

unknown. Although difficult to standardize and generally perform over 14 days, the CFU assay, which requires that viable cells multiply and differentiate, is considered by many to be the best measure of CB potency. The importance of CFU dosing on clinical outcomes after CBT was first reported by Migliaccio et al. (2000) who observed that the pre-cryopreservation CFU dose better predicted neutrophil and platelet engraftment as compared to pre-cryopreserved TNCC. Post-thaw CFU content was reported by Wall et al., on behalf of the COBLT study, to best predict engraftment and OS at 2 years (Wall et al. 2005). Extending the findings of Prasad et al. (2008), we observed in a cohort of 435 primarily pediatric patients receiving single CBT that higher CFU dosing was the only pre-cryopreservation graft characteristic predictive of neutrophil ($p = 0.0024$) and platelet engraftment ($p = 0.0063$) in multivariate analysis. Likewise, post-thaw CFU content best predicted neutrophil and platelet engraftment (both $p < 0.0001$) (Page et al. 2011a). Recently, Castillo et al. demonstrated that the clonogenic efficiency (defined as the post-thaw CFU/pre-freeze $CD34^+$) along with viable $CD45^+$ cell dose was associated with faster engraftment and improved survival (Castillo et al. 2015).

Despite the ability to assess potency, the CFU assay has several issues that currently preclude its widespread use in banking and donor selection. It is a time-consuming assay that provides results weeks later. Similar to measuring $CD34^+$ content, there are also issues with standardization between laboratories (Pamphilon et al. 2013; Brand et al. 2008). Automated scoring systems and 7-day CFU assays have been developed to address these issues, and these approaches are becoming more commonly used. There have also been focused efforts to develop alternate measures of potency that would provide results rapidly. Enumeration of CFUs using a thawed contiguous segment has been shown to be a representative of the CB product and has been used to assess potency (2003).

2.7.4 Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) is an intracellular enzyme found in high concentration in HSPCs scoring positive ($ALDH^{br}$) in this flow cytometry-based assay that are viable and likely to correlate with HSPC content of a graft (Balber 2011). $ALDH^{br}$ activity strongly correlated with CFUs and with speed of engraftment in autologous transplant recipients (Lee et al. 2014; Frandberg et al. 2015; Gentry et al. 2007; Fallon et al. 2003). This suggests that $ALDH^{br}$ content of a CBU may predict potency. At the CCBB, we have examined the $ALDH^{br}$ content of fresh and thawed CB. In fresh CB, $ALDH^{br}$ correlates well with TNCC, CFU, and $CD34^+$ content (Page et al. 2011b). However, potency of a CB graft is best assessed on the thawed product thereby reflecting any potential injury incurred due to cryopreservation and thaw. Therefore, we developed a potency assay for CBU release that can be performed at the time of confirmatory testing using a segment attached to a cryopreserved CBU. The assay enumerates $ALDH^{br}$, $CD34^+$,

$CD45^+$, glycophorin A^+, viability ($7\text{-}AAD^+$), and CFUs from the thawed segment (Fig. 2.6). Our study demonstrated a strong correlation between $ALDH^{br}$ and CFUs measured on the segment ($r = 0.78$). However, the correlation between $CD34^+$ (as a percentage of viable CD45 cells) and CFUs was weaker ($r = 0.25$).

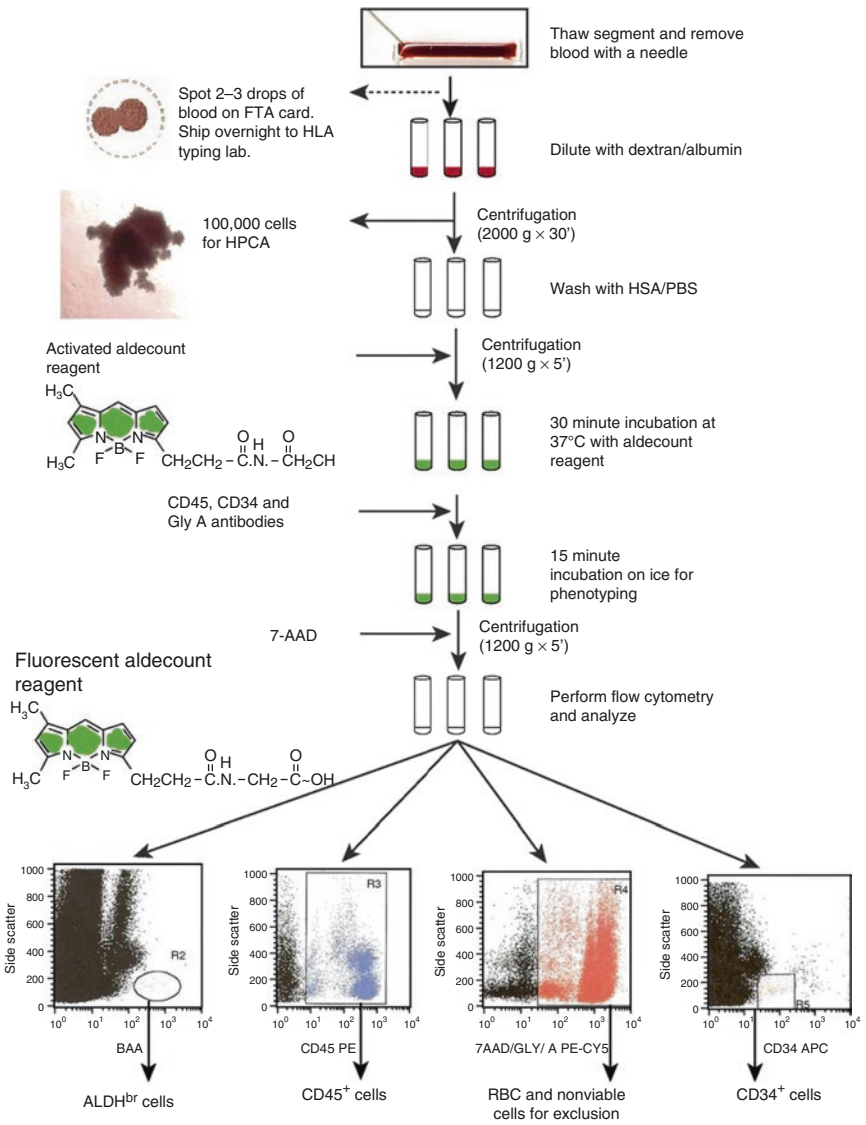

Fig. 2.6 Flowchart of the ALDH potency assay performed on attached segments of CBUs requested for CT for donor selection. *7-AAD* 7-aminoactinomycin D, *FTA* fast technology analysis, *GlyA* glycophorin A, *HSA/PBS* human serum albumin/phosphate-buffered saline, and *HPCA* hematopoietic progenitor cell assay (Used with permission) (Shoulars et al. 2016)

Comparisons between cryopreserved segments and entire unit demonstrated strong overall correlation ($r = 0.88$). We also observed faster engraftment in patients who received CB grafts with higher $ALDH^{br}$ measured on the segment ($p = 0.03$). Our findings have demonstrated that the assay can serve as a surrogate for post-thaw measurements to assess potency of a potential CBU graft. Based on these findings, we have been using this assay prior to releasing CBUs from the CCBB to the transplant centers.

2.7.5 Sterility

To prevent potential transmission of microbial agents to transplant recipients, all CB banks perform sterility assays on samples of processed cord blood units obtained prior to cryopreservation. Screening units for bacterial and fungal contamination is most commonly performed using automated culture systems with high detection capabilities (Khuu et al. 2006; Akel et al. 2013). Reported rate of contamination in the literature have been variable but generally range from 2% to 5% (Kurtzberg et al. 2005; Clark et al. 2012; Gutman et al. 2011). Considering over 13,000 processed CBUs, Clark et al. reported a contamination rate of 4%. In multivariate regression models, collections performed by OB staff, as contrasted with trained, dedicated collection staff, or after vaginal, as opposed to C-section delivery, had higher contamination rates in their series. Not surprising, vaginal and skin flora were the most common contaminates reported in the literature (Clark et al. 2012). To limit the use of CB volume for testing, investigations using pediatric culture bottles (smaller sample volume) or using processing by-products (red blood cells or plasma) have been performed. The use of pediatric culture bottles or plasma as a test sample was associated with high false-negative rates. Therefore, mixtures of RBCs and plasma spiked into adult culture bottles are considered standard practice (Ramirez-Arcos et al. 2015). CBUs screening positive in sterility assays are excluded from public bank registries. However, directed donor units that remain the best donor for a related patient which are contaminated with bacteria may be stored and used for transplantation after the recipient is covered with appropriate antimicrobial antibiotics before and after the infusion.

2.8 Finances of Public CB Banking

Public CB banking is an expensive, time-intensive endeavor. The nature of CB banking is such that extensive resources are required up front to collect, process, cryopreserve, and bank CBUs. The inventory of CBUs must be of sufficient size to provide high TNCC units representing a wide range of HLA types. Costs are partially recouped when CBUs are procured for transplantation. For most public CB banks, this is the primary source of income. Government or philanthropic

support is available for a small portion of public banks, but this may not be sufficient or reliable sources of funding. Therefore, public banks are facing a challenge to be financially self-sustainable. Individual banks can examine their practices to identify potential areas where costs can be minimized; however, many costs required for operations (i.e., supplies, equipment, and other capital costs) are fixed in nature. Since significant funds are dedicated to personnel, especially collection staff, the CCBB recently evaluated the various staffing models used at our collection sites. We found that collections performed by trained CB staff are more likely to be banked (35% *vs.* 18% of collections performed by OB staff). However, this benefit is offset by the fact that OB staff collections are more economical with respect to personnel costs. Despite this, our "best" collection site (i.e., highest proportion of collected units being banked) is fully staffed with CB bank personnel. At this site, the higher number of banked units offset the increased costs associated with additional personnel. Ultimately, individual CB banks must tailor staffing models to their own needs and the needs of their collection sites.

The banking community has actively been discussing a policy change which could lead to cost savings. The US public banking experience which provides unrelated donors for patients undergoing HCT is useful to illustrate this approach. Currently, public banks participating in the NCBI list CBUs with a minimum pre-cryopreservation TNCC of 0.9×10^9 cells. The resulting inventory contains CBUs with a median TNCC of 1.2×10^9 cells. In comparison, the median TNCC of CBUs selected for transplantation is 1.8×10^9 cells (Bart et al. 2012). Therefore, only a small portion of the inventory is likely to be selected for transplantation. It has been proposed that financial resources could be better utilized if only high TNCC units were processed and banked. Magalon et al. recently modeled a concept using registry-based data and concluded that increasing the minimum TNCC required for banking to 1.8×10^9 would be the most cost-effective strategy (Magalon et al. 2015). Our own data demonstrated that such an approach decreases operating costs but would also result in a less racially/ethnically diverse inventory (Page et al. n.d.). Until definitive data is available to show that a higher degree of HLA matching is not needed to optimize outcomes, it is important that inventory diversity is considered in this discussion. Furthermore, our data demonstrated that while these units may contain higher TNCC content, this does necessarily reflect the health or potency of the unit (Fig. 2.7). Therefore, while increasing the minimum TNCC may be a cost-effective strategy, the impact on inventory diversity, quality, and potency needs to also be considered. It is also possible that new cellular therapies using CB that are in preclinical or early phase clinical trials may provide another avenue for smaller CBUs to be used from the inventory, but this is difficult to accurately estimate. Ex vivo CB expansion techniques, discussed in an accompanying chapter, may allow for improved utilization of smaller units. In the interim, public CB banks need to explore methods, individually and collaboratively, to remain financially self-sustainable.

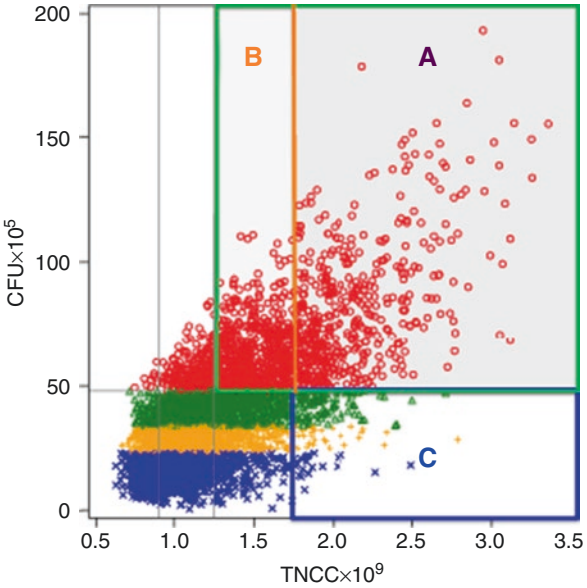

Fig. 2.7 Considering the potency (CFU content) along with post-TNCC in donor selection. The relationship between post-TNCC and the CFU content (i.e., potency) is shown for the study cohort (n = 5267 CBUs). The *vertical lines* represent (from *left* to *right*) the minimum post-TNCC required for banking (0.9×10^9), an intermediate post-TNCC (1.25×10^9), and the median post-TNCC of CBUs selected for transplantation (1.75×109). Quartiles for the CFU content are also represented (upper quartile *red circle*, second quartile *green triangle*, third quartile *gold* +, lower quartile *blue* ×). Region *A* refers to CBUs with post-TNCC of greater than 1.75×10^9 and CFU in the highest quartile. Region *B* refers to post-TNCC of 1.25×10^9 to 1.75×10^9 with CFUs in the highest quartile. Region *C* refers to CBUs with post-TNCC of more than 1.75×10^9 and CFUs in the lower three quartiles (Used with permission) (Page et al. 2014)

2.9 Family (or Private) Banking

Family banks (or private) provide an option for families wishing to store CB for personal use and willing to pay an up-front and yearly fee. Generally, these banks are "for profit" businesses and often advertise heavily to pregnant women and their providers. In actuality, the likelihood of using a privately banked CB for transplantation is quite low (Ballen et al. 2008a). Therefore, the American Academy of Pediatrics (AAP), ACOG, American Society of Blood and Marrow Transplantation, and other similar organizations worldwide do not currently recommend banking CB for personal use in a typically healthy family (Lubin and Shearer 2007; ACOG Committee Opinion No. 648: Umbilical Cord Blood Banking 2015; Ballen et al. 2008b). Guidelines from these groups stress the importance of providing pregnant women with unbiased information regarding all banking options. An exception to these guidelines is families with a history of disease (e.g., malignancy or hemoglobinopathy) that is amenable to HCT. Outcomes of CBT using sibling donors have

overall been quite successful (Gluckman et al. 2011; Screnci et al. 2016), and, therefore, banking of related CB is indicated. To facilitate this, many public and family banks offer "directed donor" programs that waive any associated banking fees. While directed donation is indicated in limited settings, continued advances in cellular therapies will likely lead to expanded indications.

Currently, family banks are not subjected to the same regulatory oversight as public banks although this varies between countries. Family banks generally use less stringent criteria for banking leading to wide variations in volume and TNCC of the private inventory. In our study of autologous CB infusions to treat acquired brain injury, CBUs from family banks were inferior to those stored in public banks with respect to collection volume, TNCC, and $CD34^+$ count (Fig. 2.8) (Sun et al. 2010). Since families bank their child's CB as "medical insurance," it is important that these banks do their utmost to ensure quality of the banked units. However, the changing landscapes of CB transplantation and regenerative medicine will likely change the indications and criteria a CBU must meet for use. In response to these changes, the role of regulatory oversight in family CB banking will need to be defined further.

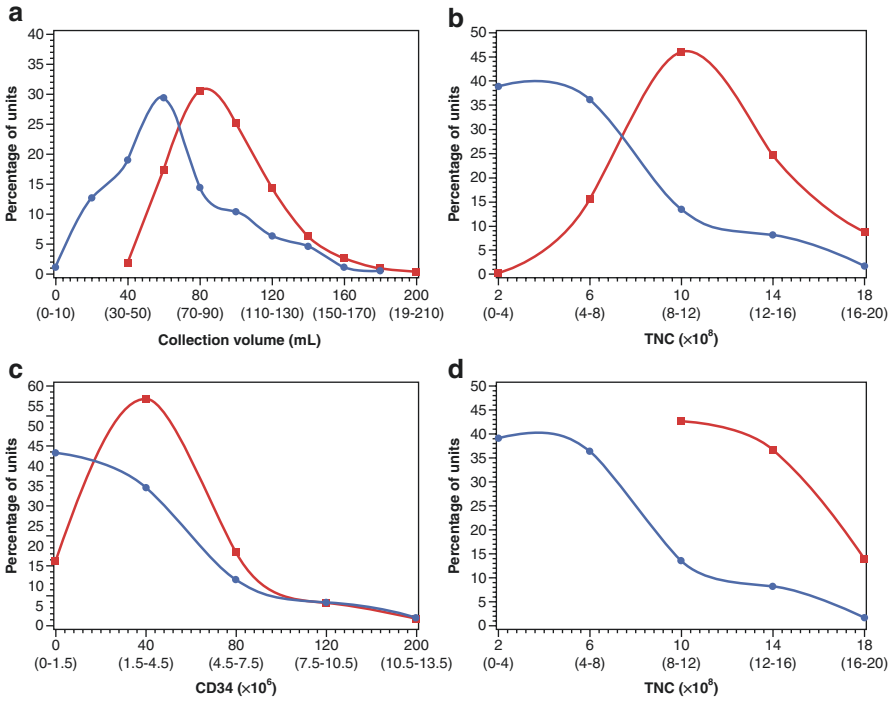

Fig. 2.8 Distributions of quality variables. In panels (**a**–**c**), the distribution of autologous CBUs is compared to the entire Carolinas Cord Blood inventory with respect to collection volume (**a**), TNC (**b**), and CD34 content (**c**). In panel (**d**), TNC of autologous CBUs [represented as *red* •] and NCBI-eligible Carolinas Cord Blood Bank CBUs [represented as *blue squares*] are compared (Used with permission) (Sun et al. 2010)

Conclusions

As the fields of CB banking and transplantation have matured into an established therapy, focus has turned to refining the use of CB for HCT and developing novel indications in the emerging field of regenerative medicine. Promising results in clinical trials using ex vivo expansion technologies will further enhance CBT and may provide an avenue for smaller units to be used. Success of these therapies relies heavily on the availability of reliable sources of healthy and potent CBUs. It is clear that CB banks are well positioned to play a major role in these exciting endeavors. However, many public CB banks are financially stressed which threatens their existence. Therefore, it is important that the banking community in partnership with regulatory bodies consider strategies that allow for financial self-sustainability while maintaining a quality and diverse inventory.

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