Chapter 1

Cord Blood Stem Cell Processing, Banking and Thawing

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Abstract

Unrelated donor cord blood (CB) is one of the three sources of hematopoietic stem cell transplantation (HSCT) that are capable of curing ~80–160 standard hematologic and certain non-hematologic indications. Despite its many advantages, the principal drawback for CB in HSCT is its limited cell dose. Our group has focused on developing minimally manipulated technologies and strategies to maximize stem, progenitor, and nucleated cell doses to overcome this limitation. The term "MaxCell" is used in this chapter to denote two proprietary CB volume reduction processing technologies that yield virtually 100% recovery of all cell lineages in the manufactured CB products, including what the authors designate as "second generation" (2nd Gen) or plasma depletion/reduction (PDR) and "third generation" (3rd Gen) MaxCB or MaxCord CB processing technologies. In our proposed nomenclature system, the traditional red cell reduction (RCR) processing techniques are designated as "first generation" methods. The properties of various popular 1st Gen techniques are compared to the MaxCell CB processing technologies. Parallel processing with the traditional hetastarch (HES) RCR technique and the patented MaxCell CB processing technology were used to compare recovery of the various stem, progenitor, nucleated, and red cell lineages. MaxCell processing technology achieved virtually 100% recovery of all stem, progenitor, and nucleated cells tested after processing, with high cell viability upon thawing. The higher cell recovery produced MaxCell inventory with higher average stem, progenitor and nucleated cell doses, allowing patients to receive CB products with higher cell doses. Clinical outcome of HSCT using MaxCell CB products was compared to the outcome of HSCT with RCR CB products published in the literature from transplant data registries or CB banks. To allow for more rigorous comparisons, two matched-pair analysis (MP) were performed using a logistic regression model to find pairs of pediatric patients with hematologic malignancies and thalassemia transplanted with RCR CB or

MaxCell CB, and patients receiving MaxCell CB showed superior engraftment, survival, and transplant-related mortality, confirming pre-match observations.

In addition, the three main post-thaw manipulation methods are reviewed. Comparison data for some of the thaw methods are presented, and matched-pair analysis was used to confirm the superiority of direct infusion thaw method over post-thaw wash for MaxCell CB products.

Keywords: cord blood banking, cord blood processing, MaxCell cord blood processing, MaxCB cord blood processing, stem cell processing, cord blood thawing, cord blood cryopreservation, cord blood transplantation, 2nd and 3rd generation cord blood technologies

1. Introduction

Today, hematopoietic stem cell transplantation (HSCT) can be performed using stem cells derived from three sources: bone marrow (BM), peripheral blood (PB), and umbilical cord blood (CB) from autologous, related allogeneic, or unrelated allogeneic donors. Autologous HSCT is not indicated for most indications, and only about one-third of patients in need of HSCT have a suitable related donor. Although there are currently tens of millions of adult volunteer donors registered worldwide, about 60% of patients will not find a suitably HLA-matched unrelated adult donor, and thus cannot access HSCT [1]. For minority patients, the probability of finding an HLA-matched unrelated adult donor is further reduced. Since the search process for an adult donor often takes several months, a significant proportion of patients will become higher risk or ineligible for HSCT or even die while waiting for the donor search [2]. In contrast, without the possibility of last minute donor refusal, CB products are available upon request and can be shipped to any transplant center in the world immediately. Importantly, cord blood transplantation (CBT) is associated with a lower incidence and severity of graft-versus-host disease (GvHD), and a partial HLA match between the donor and recipient is tolerable [3], making it an ideal donor source for minority patients without large adult donor registries.

Compared with historical controls of BM or PB HSCT, CBT has shown favorable clinical outcome despite significantly worse HLA match [3]. In the pediatric setting, CBT can be now considered established practice. For adults, CBT adoption has been slower because of cell dose limitation. Many strategies focused on overcoming this limitation are being developed, including double CBT, the utilization of two CB unit grafts [4–6], the combination of an unrelated CB graft with haplo-identical donor stem cells [7], and foregoing the post-thaw wash with direct infusion or reconstitution/dilution, which have shown promising results [8, 9]. Engraftment and survival appear to be at least equivalent to or may even be superior to historical data using post-thaw wash for CB products versus when CB was not washed. One

area which our group has focused on has been the optimization of the entire CB banking process, and in particular, the CB processing and thaw manipulations.

Almost all the published literature on CB processing has shown significant cell losses with various volume-reduction red cell reduction (RCR) processing techniques (referred to in this chapter as first generation or 1st Gen), such as the hydroxyethyl starch (hetastarch or HES) sedimentation technique, Optipress II, AXP-Express, PrepaCyte, Sepax etc.,…, with the range of loss generally around 25% for total nucleated cells (TNC) and with commensurate or higher CD34+ progenitor and colony-forming unit (CFU) loss [10–17]. As this is one of the areas that CB banks (CBBs) can have an impact, Chow has developed two novel MaxCell CB processing technologies to improve cell doses of CB products—plasma depletion/reduction processing without the reduction of red blood cells (RBC) or second generation (2nd Gen) [18] and the MaxCord or MaxCB third generation (3rd Gen) technology which combines the advantages of 1st Gen and 2nd Gen, without any of the associated disadvantages. While the processing methods and cryopreserved products are distinctly different, the cellular composition of the final infused product is identical between the two MaxCell technologies in the preferred embodiment of 3rd Gen.

Another area which has significant impact on CB potency and clinical outcome is the thaw and post-thaw manipulations of CB products. Transplant centers which do not follow CBB's prescribed and validated procedure for thawing risk decreased viability of the CB product for their patients, delayed engraftment, graft failure, transplant-related mortality, and the potential for severe adverse events (SAE) in rare cases due to thaw and Dimethyl sulfoxide (DMSO) toxicity mediated release of free hemoglobin, red cell and white cell lysates, chemokines and cytokines that may cause hemodynamic destabilization. Some of the recent development and data are reviewed.

While every step from collection to infusion of CB impacts engraftment and ultimately patient survival, in this chapter, we will focus on the roles of processing and post-thaw manipulations.

2. Processing

The world's first CB banks were established at the New York Blood Center, Düsseldorf and Milan in 1992 [13]. Initially, CB was processed and cryopreserved as whole cord blood, which we refer to in this chapter as zero generation process [19]; however, in 1995, Rubinstein et al. pointed out that "the first problem is that stockpiling ("banking") a sufficiently large number of cryoprotected Whole PCB (placental cord blood) units requires vast amounts of costly storage space in liquid nitrogen (LN). To establish an adequate panel, therefore, the hematopoietic cells of PCB units need to be concentrated into units of much smaller volume."[13] It was for this reason that volume reduction processing of CB units was developed, with associated reduction of the 'bulk of the erythrocytes and plasma'. It was reported that the red blood cell (RBC) reduction processing method increased the number of units that could be stored in the same freezer space by as much as 10-fold, and thus provided significant economic advantage [13].

Rubinstein et al. reported in 1998 the first comparison of engraftment rates using RBC-replete units (whole CB) and RBC-reduced (RCR) units [19]. In their **Table 2**, they indicated that the majority of patients in their series (337 of 546 patients) were transplanted with whole CB that were RBC-replete, and of the 365 patients (out of 546 patients) who had myeloid engraftment, the Kaplan-Meier engraftment rate was 80% [95% confidence interval (CI) 75–85%] among the RBC-replete CB transplant recipients, and 82% with RCR units (95% CI 76–88%). Whether the unit used for the transplants was RBC-replete or RCR was not considered among the variables associated with the engraftment- and transplantation-related events.

Many volume reduction methods of processing umbilical CB which reduce RBCs prior to the cryopreservation have been considered since then (**Table 1**); however, the most common method for processing CB units today is the hetastarch or HES RBC reduction (RCR), whether manual or automated. For the sake of convenience, we will call these first generation (1st Gen) cord blood processing techniques. It should be noted that these methods are often erroneously referred to as red cell depletion when all of these techniques retain considerable RBCs and do not deplete, but only reduce the number of RBCs.

Unfortunately, all 1st Gen RCR processing methods lose significant numbers of nucleated cells, stem cells, and progenitor cells as measured by CD34+ cells or colony forming units (CFU) enumeration, with an approximately 25% nucleated cell loss on average among various reports in the literature [10–17] and a range of 14–42% for the various methods reported by Takahashi et al. [10]. This is of concern because it is well-known that the success of CBT is critically dependent on cell dose, and insufficient cell dose is widely regarded as the most important limitation for umbilical CB transplantation, especially for adults and large children [1, 3]. **Table 1** lists some of the most common 1st Gen CB processing techniques.

	Technique	**Manual/automated processing**	**RBC reduction**	**Plasma depletion/ reduction**
Zero generation	Whole cord blood	Manual	None	None
First generation hetastarch	Red cell reduced	Manual SEPAX* or AXP-Express*	Yes	Yes
First generation PrepaCyte	Red cell reduced	Manual	Yes	Yes
First generation Top & bottom Optipress II	Red cell reduced	Manual	Yes	Yes
First generation Ficoll	Red cell reduced	Manual	Yes	Yes
MaxCell (MC) Technologies				
Second generation	Plasma depleted/ reduced	Manual SEPAX	No	Yes
Third generation	MaxCord RBC Reduced + RBC Replete	Manual SEPAX AXP-Express	Yes/No	Yes

* Proprietary Technologies: SEPAX from BioSafe; AXP-Express from Thermogenesis; MaxCell 2nd Gen from StemCyte; MaxCell 3rd Gen from CyteTherapeutics, Inc.

Table 1. Technical comparison of some of the most popular 1st Gen CB processing techniques and the proprietary 2nd and 3rd MaxCell CB processing technologies.

To maximize CB cell dose, R. Chow has developed two proprietary alternative CB volume reduction technologies (2nd Gen and 3rd Gen) which he calls MaxCell CB processing technologies, because both share the common characteristics of (1) depleting or reducing plasma, (2) maximizing stem cell, progenitor, nucleated and mononuclear cell recovery after processing and most importantly, and (3) having identical cellular composition upon infusion for one version of the third generation technology (**Tables 1–3**).

Post-processing recovery cell # recovery %	Volume (ml)	Frozen volume (ml)	RBC (ml)	DMSO (g)	5% Dextran-40 (ml)	6% HES (ml)	CPD—Plasma (ml)
RCR	20–28	25–35	Varies	1.25–1.75	2.3–3.5	<10, if present	Varies
Gen 1-HES			1–15				4–15
MaxCell	40–84	50–100	28–70	5.0–10.0	10–20	0.0	10–12
Gen 2							
MaxCell	20–28	25–35	1–15	1.25–1.75	2.3–3.5	<10, if present	4–15
Gen 3	40–84	50–100	28–70	5.0–10.0	10–20		10–12

1st generation RBC-reduced CB Products—Manual: Hetastarch most commonly used; however, also include PrepaCyte, Optipress, Ficoll Hypaque; Automated: Sepax & AXP-AutoXpress.
MaxCell: 2nd generation plasma depleted/reduced CB products and 3rd generation MaxCord CB products.

Table 2. A comparison of the volumes and amount of some of the various components of first (hetastarch), second and third generation CB technologies.

The MaxCell second generation (2nd Gen) technology or plasma depletion/reduction reduces or depletes plasma without reducing RBCs. Plasma is removed or reduced from the product prior to the cryopreservation [18]. Although the resulting volume of CB products processed by such a method is two to three times larger, 2nd Gen results in greater than 99.9% processing recovery of nucleated cells and all critical cell types prior to the testing and archival sampling. True nucleated cell loss was less than 0.01% from 2nd Gen CB processing, as evidenced by complete blood count (CBC) enumeration of the discarded plasma fraction. In practice, because of the testing and archival sampling, the actual loss will be raised to around 5–10% of the collected CB cell dose. Such minimal loss was reproducible from validation to an actual MaxCell inventory of more than 12,000 CB products that had pre- and post- processing CBC available. Even after thawing, in 188 post-thaw segment samples, a median of 90.33% *post-sampling/post-thaw* TNC recovery and a median of 91.84% *post-sampling/post-thaw* CD34 cell recovery of preprocessing values were achieved for 2nd Gen CB products. The resultant volume, DMSO load, RBC, WBC and RBC lysate content of 2nd Gen CB products are similar to cryopreserved peripheral blood products (**Tables 1–3**).

The newest MaxCell technology, MaxCord processing technology, referred to as third generation (3rd Gen) CB processing technology in this chapter, combines the advantages of ease and convenience of thawing for the 1st Gen products and maximum stem (VSEL), progenitor (CFU

and CD34+), nucleated, and mononuclear cell recovery of the 2nd Gen, without any of the associated disadvantages. While the processing methods and cryopreserved products are distinctly different, with the preferred embodiment of 3rd Gen technology, the cellular compositions of the final infused products are virtually identical between the two MaxCell technologies. **Table 2** outlines some of the component differences in volume, RBC content, and amount of additional components, such as DMSO, dextran-40, HES, and anticoagulant CPD.

Post-processing Recovery Cell # Recovery %	**RBC ($\times10^{10}$)**	**Total nRBC ($\times10^{7}$)**	**Total MNC ($\times10^{7}$)**	**TNC ($\times10^{7}$)**	**CD34+ cells ($\times10^{6}$)**	**Viability (%)**	**CFU-GM ($\times10^{5}$)**	**CFU-E ($\times10^{5}$)**	**CFU-GEMM ($\times10^{5}$)**	**Total CFU ($\times10^{5}$)**	**Total VSEL ($\times10^{3}$)**
RCR	8	2.1	26	65.77	1.16	96%	3.5	3.6	4.6	11.7	4.434
Gen 1- HES	32.9%	78.1%	80.7%	78.7%	84.0%	98.3%	42.1%	48.5%	50.4%	47.1%	54.6%
MaxCell	Gen 2–24	2.5	31	81.56	1.42	98%	7.8	4.7	9.3	21.8	8.134
Gen 2/3	100.2% Gen 3–8 33%	91.5%	92.4%	96.4%	99.9%	99.3%	78.1%	111.2%	88.4%	88.1%	100.1%
Gen 2 and/or 3 MaxCellGen/ RCR Recovery Ratio	**Gen 2 312%** Gen 3 100–312%	118%	117%	**124%**	**121%**	102%	**225%**	128%	**202%**	**186%**	**181%**
P value	**Gen 2 <0.0001** Gen 3 Variable	0.18	0.27	**0.002**	**0.003**	0.24	**0.05**	0.50	**0.001**	**0.01**	**0.007**

Comparisons between HES 1st Gen and MaxCell 2nd and 3rd Gen are performed using parallel processing comparison using one half of the same cord blood unit for first generation hetastarch and the other half for MaxCell CB Products [18]. Cell types which exhibit significant differences are highlighted by boldfaced fonts.
Second and third generation cord blood products have similar yield for all cell lineages, and only differ in the final RBC yield depending on the embodiment of 3rd Gen used. Third generation is similar to first generation in RBC reduction in RBC reduction. Sample size n = 10 for all the cell types, except for VSEL, which had a sample size n = 3. P values are calculated using the paired-sample *t*-test.

Table 3. Stem cell (VSEL), progenitor cell (CFU and CD34+), nucleated cell, mononuclear cell, nucleated RBC, RBC, and viability recovery of 1st (hetastarch method) MaxCell 2nd and MaxCell 3rd generation CB processing.

Third generation CB processing technology is perhaps the most flexible CB processing technology developed to date, with the additional option to reduce RBCs without losing as much stem, progenitor, nucleated, and mononuclear cells as 1st Gen RCR techniques (**Table 3**). **Table 1** highlights some of the processing technical differences among various popular 1st Gen techniques versus 2nd Gen and 3rd Gen CB processing technologies. Unlike Chow's 2nd Gen CB technology which is only amenable to SEPAX-automated processing and not accessible to AXP-Express, Chow's latest MaxCell technology can be

automated using both SEPAX or AXP-Express. **Table 3** shows the differences in CB stem cell (VSEL), hematopoietic progenitor cell (CD34+ and CFUs), total nucleated cells (TNC), mononuclear cells, nucleated RBCs (nRBC), RBCs, and viability between the 1st Gen HES technique and the proprietary MaxCell 2nd/3rd Gen technologies. While the processing methods and cryopreserved products are distinctly different, the cellular composition of the final infused product is identical between the two MaxCell technologies using the preferred embodiment of 3rd Gen (**Table 3**) technology, while other embodiments can be adapted to reduce RBC, free hemoglobin, DMSO, and WBC lysate load for certain situations to better suit the needs of the individual patients (**Table 2**).

Barker et al. [20] have questioned whether the MaxCell data [8] indicating significantly higher TNC doses with red cell-replete MaxCell CB products are meaningful. Indeed, it was speculated that a 'correction factor' of 0.75 should be applied to the reported TNC content of RBC-replete products, arguing that such products contain the noncritical nucleated RBC (nRBC) and neutrophils, and implying that critical stem and progenitor cells are somehow not retained to a similar extent as TNC. Unfortunately, no hard data accompanied this implication or their recommendation and the authors ignored the data on tens of thousands of CB units presented by Chow et al. [18] in **Table I–IV** of their paper, which showed significantly higher recovery of stem (VSEL), progenitor (CD34+ and CFU), nucleated and mononuclear cells with parallel processing of the two halves of the same CB units using MaxCell 2nd Gen technologies versus RCR techniques. More importantly, the superior outcome data for the MaxCell CB products [8] were similarly disregarded. Petz and Chow [21] pointed out that implementation of Barker's recommendation, if incorrect, would underestimate the progenitor cell content of RBC-replete CB products, resulting in the inappropriate rejection of certain optimal units by transplant physicians in favor of RCR products with an apparently higher TNC dose after application of the 'correction factor' for RBC-replete products.

	1st generation RCR	2nd/3rd generation MaxCell
Neutrophil ANC500 engraftment	~75–80%	~85–90%
Overall survival	~40–45%	~57–75%

This outcome assumes that large cord blood banks have normal and similar distribution of patients, disease indications, and disease stage.

Table 4. Published overall clinical outcome of CBT patients receiving RCR CB products from NYBC, COBLT, NMDP, and CIBMTR [19, 23–34] versus MaxCell CB products from CIBMTR audited outcome data [8].

To study this issue, Chow et al. divided 10 CB units in half and processed one half by RCR using the most commonly used hetastarch method, and the other half by MaxCell in parallel [18]. As indicated in **Table 3**, the average TNC of MaxCell CB products after processing was 124% of that in RCR units ($p = 0.002$). Moreover, MaxCell caused virtually no loss of CD34 cells compared to RCR produced CB, with the mean post-processing CD34 cell count in MaxCell products being 121% of that in RCR units ($p = 0.003$). Page et al. [22] have reported on their experience with 435 CB transplants and concluded that total CFU is the best predictor

of engraftment. Of major importance is that the mean recoveries of CFUs were also higher for MaxCell products [11], as shown in **Table 3**, 225% for CFU-GM ($p = 0.05$), 202% for CFU-GEMM ($p = 0.001$) and 186% for total CFU ($p = 0.01$). Taken together, this means that CFU, the most important cellular predictors of engraftment success, showed an even bigger difference than TNC between RCR and MaxCell products, in contrast to the speculation by Barker and NYBC [20].

In fact, in clinical studies of MaxCell CB products, outstanding clinical outcome in terms of engraftment and patient survival have been achieved. **Table 4** showed published engraftment and survival outcome for transplantation using 1st Gen RCR CB products from diverse CB banks (NYBC, EuroCord, COBLT CBB, London CBB, French CBB) or large outcome data registries, such as CIBMTR or NMDP [19, 23–34] versus 2nd/3rd Gen Max Cell CB products [8].

Ballen et al. [34] studied the outcome of manually processed RCR CB products, automated processed RCR CB products, and manually processed MaxCell 2nd Gen CB products. While acknowledging some of the significant design flaws in their study, the authors concluded that automated processing systems resulted in higher day-28 neutrophil recovery than manually processed plasma-reduced products; however, overall survival by day-100 was not different among the three groups. Many study decisions and design issues were not clearly explained or delineated in this study, such as patient selection or study end point selection. For patients receiving Max Cell 2nd Gen CB products and manually processed RCR CB products, there were only 133 and 279 patients, respectively, when hundreds to thousands more were available fitting the study criteria during the study period for both of these groups. For example, even in a 2008 study with just pediatric hematological malignancy patients from one transplant center (U. Arizona) and one CBB (StemCyte), Graham et al. presented data for 105 matched pairs of patients from 128 patients transplanted with PDR 2nd Gen CB and 112 patients transplanted with RCR 1st Gen CB [35]. In fact, it is obvious that of the 16 public CB banks in the study, at least four of the banks each alone had more patients fitting the exact criteria for patients transplanted with manually processed CB in the study, so the reasons why these other patients were not selected and the reasons for inclusion of the particular patients in the study were not clear. The decision to use only 100-day survival, and not 1-year, 3-year, or 5-year overall survival, was also quite unconventional. All three patient groups had approximately 80% 100-day overall survival, which should not give an erroneous impression that long-term survival is that high or that long-term survival may be the same for all three groups. Previous studies with far larger patient populations not restricted to acute leukemia/MDS from NYBC, COBLT, CIBMTR, and NMDP have confirmed that transplants with RCR CB yielded around 40–45% 1-year survival and much higher 100-day transplant-related mortality [19, 23–33]. Indeed, long-term survival data on many more patients fitting the study criteria were available from CIBMTR, NMDP, and the various banks when the study was analyzed, so it was odd that 100-day overall survival (OS) was chosen as one of the study's primary end points, when it would have been far more meaningful and just as easy to calculate and show longer term survival and transplant-related mortality data, as is typical for such CIBMTR studies. In fact, our experience of a large MaxCell CB bank

with thousands of patients transplanted with its products have shown that survival or transplant-related mortality during the first 100 days is often not predictive for long-term survival or patient mortality [8, 35–43].

The most rigorous method to address clinical outcome differences between different types of CB processing methods would be to conduct prospective double-blind placebo-controlled clinical trials; however, in HSCT, this is largely not feasible. Instead, matched-pair analyses allow for comparisons that are reasonably free from extraneous factors. To investigate rigorously whether clinical outcome differences exist between RCR and MaxCell CB products, Chow and collaborators performed matched-pair analysis using retrospective outcome data. Moreover, they secured an independent third party's assistance from CIBMTR to audit the outcome data with patient chart review on site at transplant centers using an audit design proposed by CIBMTR and finding the MaxCell CB outcome data to be 97.3% accurate, with no errors in survival, mortality, or engraftment. For both studies, all thalassemia and pediatric hematological malignancy patients were included and outcome were similar before and after matched-pair comparisons. A logistic regression model was used to find patients with similar characteristics to form pairs. For both studies, cumulative incidence was used for ANC500 neutrophil and platelet 20K and 50K engraftment. Kaplan–Meier was used for overall survival, disease-free survival, transplanted-related mortality, and relapse or autologous recovery. Cox regression analysis, log-rank test, univariate comparisons and the paired Prentice-Wilcoxon method were performed to compare the two matched-pair groups. Using the above methodologies, two rigorous retrospective matched-pair analysis of patients using RCR versus MaxCell CB products were conducted for thalassemia as well as for pediatric hematological malignancy patients [35–38].

	RCR 1st Generation CB	**MaxCell 2nd/3rd generation CB**
1-Yr Overall Survival	**Matched Pair 53 ± 18 %**	**Matched Pair 96 ± 4 %**
Thalassemia Matched Pair	Univ. RR = 0.09; $p = 0.03$	Univ. RR = 0.09; $p = 0.03$
	PPW Test $p = 0.001$	PPW Test $p = 0.001$
1-Yr Disease-Free Survival	**Matched Pair 40 ± 15 %**	**Matched Pair 89 ± 6 %**
Thalassemia Matched Pair	Univ. RR = 0.17; $p = 0.01$	Univ. RR = 0.17; $p = 0.01$
30 Pairs	PPW Test $p = 0.0001$	PPW Test $p = 0.0001$
1-Yr Transplant-Related Mortality	**Matched Pair 47 ± 18 %**	**Matched Pair 4 ± 4 %**
Thalassemia Matched Pair	Univ. RR = 0.09; $p = 0.03$	Univ. RR = 0.09; $p = 0.03$
30 pairs	PPW Test $p = 0.001$	PPW Test $p = 0.001$

Relative Risk calculated by Cox Regression for Univariate Analysis, with Red Cell Reduced CB products as reference. PPW = Paired Prentice-Wilcoxon test used in comparing matched-pair patients [**35–38**]. Absolute Neutrophil Count (ANC 500) is the first day of 3 consecutive days of an absolute neutrophil count equal to or greater than 0.5×10^9/L prior to day 60 after transplantation (ANC 500)) engraftment. Platelet 20K engraftment is the first day of 7 consecutive days of a platelet count equal to or greater than 20×10^9/L prior to the day 180 after transplantation.

Table 5a. Matched-Pair Comparison of Clinical Outcome of Patients transplanted with First Generation Red Cell Reduced CB Products versus MaxCell 2nd/3rd Generation CB products—30 pairs of thalassemia patients [36, 38] (Jaing et al. 2008) and 105 pairs of pediatric malignancy patients [35, 37, 38] (Jaing et al. 2008).

	RCR 1st Generation CB	**MaxCell 2nd/3rd Generation CB**
ANC500 Neutrophil Engraftment	85 ± 6 %	89 ± 6%
Pediatric Leukemia Matched Pair	Univ. RR = 0.81; $p = 0.06$	Univ. RR = 0.81, $P = 0.06$
92 pairs	Log-Rank Test $p = 0.12$	Log-Rank Test $p = 0.12$
	Matched Pair 87 ± 6 %	Matched Pair 91 ± 7%
	PPW p=NS	PPW p=NS
Platelet 20K Engraftment	**55 ± 6 %**	**69 ± 6 %**
Pediatric Leukemia Matched Pair	Univ. RR = 1.60; $p = 0.007$	Univ. RR = 1.60; $p = 0.007$
	Log-Rank Test $p = 0.006$	Log-Rank test $p = 0.006$
	Matched Pair 56 ± 6 %	**Matched Pair 71 ± 7 %**
	Matched Pair PPW $p = 0.001$	Matched-Pair PPW $p = 0.001$
Platelet 50K Engraftment	**51 ± 6 %**	**65 ± 5 %**
Pediatric Leukemia Matched Pair	Univ. RR = 1.41; $p = 0.06$	Univ. RR = 1.41; $p = 0.06$
	Log-Rank Test $p = 0.05$	Log-Rank Test $p = 0.05$
	Matched Pair 52 ± 6%	**Matched Pair 68 ± 7 %**
	Matched-Pair PPW $p = 0.007$	Matched-Pair PPW $p = 0.007$
1-Yr Overall Survival	**48 ± 5 %**	**61 ± 4 %**
Pediatric Leukemia Matched Pair	Univ. RR (death) = 0.74; $p = 0.01$	Univ. RR (death) = 0.74; $p = 0.01$
	Log-Rank Test $p = 0.11$	Log-Rank Test $p = 0.11$
	Matched Pair 50 ± 5%	**Matched Pair 68 ± 5%**
	Matched-Pair PPW $p = 0.005$	Matched-Pair PPW $p = 0.005$
1-Yr Disease-Free Survival	46 ± 5 %	54 ± 4 %
Pediatric Leukemia Matched Pair	Univ. RR (death) = 0.86; $p = 0.11$	Univ. RR (death) = 0.86; $p = 0.11$
	Log-Rank Test $p = 0.41$	Log-Rank Test $p = 0.41$
	Matched Pair 47 ± 5%	Matched Pair 61 ± 6%
	Matched Pair PPW $p = 0.07$	Matched Pair PPW $p = 0.07$
1-yr Transplant-Related Mortality	**45 ± 5 %**	**23 ± 4 %**
Pediatric Leukemia Matched Pair	Univ. RR = 0.49; $p < 0.001$	Univ. RR = 0.49; $p < 0.001$
	Log-Rank Test $p = 0.002$	Log-Rank Test $p = 0.002$
	Matched Pair 44 ± 5%	**Matched Pair 17 ± 4%**
	Matched-Pair PPW $p = 0.0001$	Matched-Pair PPW $p = 0.0001$

Relative Risk calculated by Cox Regression for Univariate Analysis, with Red Cell Reduced CB products as reference. PPW = Paired Prentice-Wilcoxon Test used in comparing matched-pair patients [**35–38**]. Absolute Neutrophil Count (ANC 500) is the first day of 3 consecutive days of an absolute neutrophil count equal to or greater than 0.5×10^9/L prior to day 60 after transplantation (ANC 500)) engraftment. Platelet 20K engraftment is the first day of 7 consecutive days of a platelet count equal to or greater than 20×10^9/L prior to the day 180 after transplantation.

Table 5b. Matched-Pair Comparison of Clinical Outcome of Patients transplanted with First Generation Red Cell Reduced CB Products versus MaxCell 2nd/3rd Generation CB products—105 pairs of pediatric malignancy patients [35, 37, 38].

For the thalassemia matched-pair study, 48 patients and 10 patients transplanted with MaxCell CB and RCR CB, respectively, and 3 MaxCell CBT patients were matched to each RCR CBT patients to form 30 pairs [36, 38]. Outcome comparisons of the two patient groups pre-match showed superiority in overall survival, disease-free survival, and transplant-related mortality for patients transplanted with MaxCell CB. Factors matched between the two groups were age, weight, #HLA matches, TNC dose, and transplant center experience. As the patients are mostly pediatric, there were no differences in median TNC between the two patient groups before (MaxCell 9.1 versus RCR 8.9 × 10^7/kg) or after the match (MaxCell 9.1 versus RCR 8.9 × 10^7/kg). **Table 5** showed significant improvement in 1- to 3-year overall survival and disease-free survival and 1-year and 3-year transplant-related mortality with the use of MaxCell CB for thalassemia patients [36, 38]. Interestingly, neutrophil engraftment, and short-term (100-day) survival or transplant-related mortality were not significantly different between MaxCell and RCR CB products.

For the pediatric hematological malignancy matched-pair study, factors matched between the two groups were age, weight, #HLA matches, TNC dose, disease, and disease status [35]. Combining audited outcome data from one CBB (StemCyte) and data from one transplant center (U. Arizona), Graham et al. presented data for 105 matched pair of patients (paired from 128 patients transplanted with MaxCell 2nd Gen CB and 112 patients transplanted with RCR 1st Gen CB). **Table 5** shows that for the 105 pairs of pediatric hematological malignancy patients, 1- and 3-year overall survival, 100-day, 1-year and 3-year transplant-related mortality, and platelet (20K and 50K) engraftment were significantly improved with the use of MaxCell CB, while disease-free survival trended towards improvement [35, 37, 38]. Superior outcome of the MaxCell CB patient group in pre-match comparisons were confirmed by the results seen in the matched-pair analysis. Again, neutrophil engraftment and short-term (100-day) survival or transplant-related mortality were not significantly different between MaxCell and RCR CB products despite significant advantages in platelet engraftment, overall survival and transplant-related mortality.

Using data supplied by the NMDP for its large CB inventory of its CBB network (as of June 30, 2006) derived from almost 50,000 units (10,912 MaxCell and 38,819 RCR), Chow et al. [8] showed that a 24% superior nucleated cell recovery amplified into a 200% increase for MaxCell over RCR for the proportion of the inventories with products that had TNC counts higher than 150 × 10^7 (20% versus 10% of the inventory; test for difference in proportions, $p < 0.0001$) and a threefold difference for the proportion of products that had TNC counts higher than 200 × 10^7 (6% versus 2% of the inventory; test for difference in proportions, $p < 0.0001$). Therefore, the effectiveness of MaxCell CB processing is supported by data derived from the NMDP CB inventories of almost 50,000 units, which proves that the MaxCell CB inventories have significantly higher proportions of products with high TNC doses than the RCR CB inventories. Indeed, the MaxCell CB inventories had two to three times the proportion of high-cell dose products with TNC number of 150 × 10^7 or above and 200 × 10^7 or above ($p < 0.0001$) than the inventories of RCR units. Thus, MaxCell CB processing provides more efficient utilization of this valuable resource. This would seem to be particularly significant for those patients who participate in directed CB donation and private

banking, because of the uniqueness of the cellular content and the importance of cell dose in outcome of HSCT.

3. Thawing of cryopreserved CB products

Unlike cryopreservation, thawing should be performed as quickly as possible by immersing the body (but not the ports) of cord blood bag in a 37°C water bath to an icy slush mixture. After thawing, there will be invariably some cell lysis, including 5–20% of the RBCs [44], a certain amount of WBC, principally neutrophils which do not survive freezing and thawing well, and occasional cell clumping and viscosity due to the release of chromosomal DNA. **Table 6** shows the expected cell loss for the various 1st Gen methods [44] as well as for the MaxCell 2nd/3rd Gen technologies [8]. The published cell loss and death associated with the CB processing method is listed below in **Table 6**, which showed the least TNC, CD34, and CFU loss for MaxCell CB products, followed by PrepaCyte, and with AXP-Express coming in last of the four techniques tested by Akel et al. [44]. Screnci et al. [45] independently confirmed that 42 un-manipulated RBC-replete CB products had significantly better post-thaw and wash recovery of TNC than 36 RCR CB, 95.2 ± 14.7% versus 85 ± 15.4% ($p = 0.004$).

	Red cell reduction 1st generation				**MaxCell 2nd/3rd generation**
	Hetastarch*	Prepa Cyte-CB*	Sepax*	AXP*	MaxCell** (n = 188)
TNC	18% vs. PrepaCyte $p < 0.01$ vs. Sepax $p < 0.05$ 14%*** vs. MaxCell $p = 0.004$	10%	14%	20% vs. PrepaCyte $p < 0.001$ vs. Sepax $p < 0.01$	8.89% 4.8%***
Total MNC	8%	13%	7%	20%	<10%
CD34+ Cells	32%	24%	19%	37%	8.12%
CFU	47% vs. PrepaCyte $p < 0.05$	20%	37%	53% vs. PrepaCyte $p < 0.001$	<10%
Viable NCs by Trypan Blue	32%	28%	27%	22%	Variable depends on #neutrophils in CB

Table 6. Post-thaw cell loss vs. pre-freeze cell count [11**, 44*, 45***].

4. Post-thaw manipulations

There are generally three main methods of manipulations after thaw of CB products, with many variations among different banks and transplant centers. **Table 7** summarizes whether each method reduces the total amount or just dilutes the concentration of DMSO, free hemoglobin or WBC lysates from thawing, safe thaw-to-dilution and thaw-to-infusion times, as well as summarizes the advantages and disadvantages of each method. Automated procedures using the Biosafe Sepax system can be used on all CB product types and was shown to be as effective as manual washing in terms of cell recovery and viability [46]. The Duke group found equal TNC recovery, identical viability, among 30 Sepax and 195 manual washed products with slightly better CFU and lower CD34+ recovery for the Sepax products [47].

	Reduced total mass			**Reduced concentration**			**Safe thaw-to-dilution time** **Safe thaw-to-infusion time**		
	Reduced DMSO	**Reduced Free Hgb**	**Reduced WBC**	**Diluted DMSO**	**Diluted Free Hgb**	**Diluted WBC**	**RCR**	**MaxCell 2nd Gen**	**MaxCell 3rd Gen**
Bedside Thaw/ Direct Infusion	No	No	No	No	No	No	30 min 30 min	10 min 10 min	30 min 30 min
Dilution/ Reconstitution	No	No	No	Yes	Yes	Yes	30 min 4 hr	10 min 1 hr	30 min 4 hr
Dilution/wash	Yes	Yes	Yes	Yes	Yes	Yes	30 min 8 hr	10 min 2 hr	30 min 8 hr

Table 7a. Comparisons of the three major CB product thaw methods and the various parameters, Pros and Cons. [8, 21, 44, 48].

	Pros	**Cons**
Bedside Thaw/ Direct Infusion	*In matched-pair analysis of 258 patients forming 129 pairs of non-washed versus washed patients (95 malignancy pairs & 34 nonmalignant indication pairs), Direct Infusion of MaxCell CB resulted in IMPROVED OS, DFS, TRM, ANC 500, platelet 20K and platelet 50K engraftment, Higher Limited cGvHD but Lower Extensive cGvHD *Minimum cell loss if within safe thaw-to-infusion time	*Immediate infusion necessary, within first 10 min preferably for minimum additional cell lysis due to DMSO toxicity. Not longer than 30 min. *Highest Load & Concentration of DMSO, RBC & WBC lysate *No addition of extra stabilizing agent *Inability to assess product characteristics in the freezing bag; however, product characteristics in the attached segment can be used as a surrogate.

	Pros	Cons
	*Minimum infusion volume *Least technically challenging—with most important element being control of thaw-to-infusion time *Similar to other cryopreserved cellular product *Recommended Thaw Method for MaxCell CB Products by Manufacturer for most situations.	*Potential for AE if used improperly not according to Manufacturer's Validated & Recommended Protocol.
Dilution/ Reconstitution	*More time from thaw to infusion *Controlled Thaw in Cell Therapy Lab *Hyperosmolar re-equilibration resulted in Diluted (though Equal Amount of) DMSO, Free Hgb, RBC & WBC lysate as Direct Infusion/Bedside Thaw *Recommended Thaw Method for 1st Gen RCR CB Products by some CB banks	*Largest infusion volume of three methods *Same total mass of DMSO, Free Hgb, RBC & WBC lysate as Direct Infusion/Bedside Thaw *Initial Dilution Step should be performed preferably within the first 10 minutes, in no case longer than the first 20 minutes for MaxCell CB and 30 minutes for RCR CB. *Potential for AE if used improperly not according to Manufacturer's Validated & Recommended Protocol.
Dilution/Wash	*Removal of more than 80% DMSO and RBC and WBC Lysate *Removal of Hetastarch, PrepaCyte or other colloidal agents *Longest Safe Thaw to Infusion Time as long as the initial dilution is performed within the first 10 minutes *Recommended Thaw Method for 1st Gen RCR CB Products by some CB Banks *Recommended 1:7 Dilution-Wash Thaw Method for MaxCell CB products by Manufacturer for certain situations.	* Compared to Direct Infusion, Dilution/Wash of MaxCell CB resulted in Worse OS, DFS, TRM, ANC 500, Platelet 20K and Platelet 50K engraftment, Lower Limited cGvHD but Higher Extensive cGvHD. *Most significant cell loss of three methods during discard of post-centrifugation supernatant *Risk of bag breakage *Risk of cell aggregation with centrifugation *Longest time and highest technical complexity of three methods *Initial Dilution Step should be performed preferably within the first 10 minutes, in no case longer than the first 30 mins *Potential for AE if used improperly not according to Manufacturer's Validated & Recommended Protocol.

Table 7b. Pros and Cons of the three major CB product thaw methods [8, 21, 44, 48].

4.1. Bedside thaw/direct infusion method

The CB product is thawed at bedside using the above thaw technique and immediately administered to the patient. Thaw to completion of infusion is typically completed within 10 min, with a maximum of 20 min, to avoid DMSO-induced toxicity and lysis of cells. Cell loss and technical complexity are minimal for bedside thaw/direct infusion of the three main thaw methods, especially if the infusion bag is flushed with saline or another approved infusion fluid to rinse out and inject the residual cells. Delay in infusion after thaw will lead to DMSO toxicity and cell lysis, resulting in loss of cell viability and release of potentially harmful cytokines, chemokines and cell debris that may potentially cause adverse events if released in sufficient amount. For all of the SAE reported to NMDP for CB infusion in 2008–09, prolonged thaw to dilution or infusion (essentially *in vivo* dilution) times were the common element among all the cases (NMDP, unpublished data). Further clinical data from the St. Louis bank's transplanted CB from the same publication [48] showed that the no-wash direct infusion provided the best post-thaw TNC recovery (median 99.0%, mean 85.6%, $p < 0.01$) and viability (median 95.0%, mean 89.3%, $p < 0.01$) over no-wash dilution (TNC recovery median 78.0%, mean 78.4%, viability median 88.0%, mean 84.8%) and post-thaw wash (TNC recovery median 78.6%, mean 77.4%, viability median 73.0%, mean 74.0%) [48].

There has been some concern that the presence of residual RBC in cryopreserved MaxCell CB may adversely affect the safety of HSCT; however, lysed RBC ghosts and free hemoglobin do not usually give rise to severe problems [49] unless a patient has compromised renal function or is on nephrotoxic drugs. The rare occurrences of acute renal failures with HSCT are frequently self-limiting or resolved with dialysis. While MaxCell manufacturers have always advocated direct infusion without post-thaw washing or dilution for most patients receiving MaxCell CB products, they also caution that for children, small patients, patients with compromised renal function (pre-existing or iatrogenic), patients with known sensitivity to DMSO, RBC or WBC lysates, chemokines and cytokines, and, lastly, for transplant centers that cannot directly infuse MaxCell CB products within 10–20 min of thawing, post-thaw washing is indicated. If post-thaw reconstitution or washing is to be performed, then it is of utmost importance to dilute the MaxCell CB product adequately (serial 1:1 dilutions three times to 1:7 final minimal dilution) within 10 min of thawing and to complete infusion of the washed product within 1–2 h, respectively (**Table 7**). The lack of SAEs when MaxCell CB products are thawed using strictly either the manufacturer's direct infusion method or post-thaw washing procedures or following proper validated good thawing practices has been documented [8].

Chow et al. [50–55] showed that for MaxCell CB products, direct infusion resulted in superior 1-year and 3-year overall survival, disease-free survival, and transplant-related mortality, as well as neutrophil ANC500 and platelet 20K and 50K engraftment, with higher limited cGvHD but lower extensive cGvHD over post-thaw wash [50–54]. **Table 8** shows the results of bedside thaw/direct infusion compared to post-thaw wash methods for MaxCell CB products in a matched-pair analysis of 258 patients forming 129 pairs of non-washed versus washed patients (95 malignancy pairs and 34 nonmalignant indication pairs), which confirmed the pre-match observations that direct infusion of MaxCell CB resulted in improved neutrophil ANC500 and platelet 20K, and 50K engraftment, as well as higher 1-year and 3-year overall survival, disease-

free survival, and lower transplant-related mortality, over transplants where MaxCell CB was post-thaw washed. Lastly, higher limited cGvHD with lower extensive cGvHD of direct infusion will maximize graft-versus-leukemia effect (GvL) without the increased mortality associated with severe cGvHD [55–59].

Paired Prentice-Wilcoxon Test	ANC 500	Platelet 20K	Platelet 50K	Relapse	TRM	OS	DFS	aGvHD II-IV	aGvHD III-IV	cGvHD Ltd	cGvHD Ext
P-value	0.002	<0.0001	0.0003	0.26	0.050	0.009	0.046	0.04	0.09	<0.0001	0.02
Log-Rank Test	ANC 500	Platelet 20K	Platelet 50K	Relapse	1-Year TRM	1-Year OS	1-Year DFS	aGvHD II-IV	aGvHD III-IV	cGvHD Ltd	cGvHD Ext
NW Outcome W Outcome P-Value	89±6% d21 83±6% d28 p=0.002	78±7% d45 58±6% d56 p=0.001	71±7% d50 53±6% d64 p=0.002	--	19±4% 40±5% p=0.007	69±4% 48±5% p=0.008	61±5% 43±5% p=0.02	37±5% 32±5% p=0.50	14±4% 14±3% p=0.72	24±5% 11±3% p=0.04	4±2% 17±4% p=0.003
Interpretation	Faster & Improved ANC500 Engraftment for NW	Faster & Improved Platelet 20K Engraftment for NW	Faster & Improved Platelet 20K Engraftment for NW	--	Lower TRM for NW	Higher OS for NW	Higher DFS for NW	Higher aGvHD II-IV for NW (PPW)	-	Higher Limited cGvHD for NW	Lower Extensive cGvHD for NW

Paired Prentice-Wilcoxon test and log-rank tests were used to analyze 258 patients forming 129 pairs; (95 pairs malignancies and 34 pairs nonmalignant indications; relapse calculations are only for the 95 malignancy pairs). Paired Prentice-Wilcoxon test uses matched pairs of patients infused with post-thaw washed versus unwashed MaxCell CB products. Log-rank tests used univariate analysis of previously matched patients.

Table 8. Matched-pair analysis results comparing 129 pairs of 258 CBT patients receiving unwashed versus washed MaxCell CB products [56–59].

4.2. Dilution and wash thaw method

In 1995, Rubinstein et al. [13] described a thaw method, which consisted of slow reconstitution of the thawed unit three times with an equal volume of isotonic solution (5% [wt/vol] dextran-40/2.5% [wt/vol] human serum albumin) to an eventual ratio of 1:7 product:diluent (1:1 → 1:3 → 1:7), followed by centrifugation at 4°C at 400 × g for 10 min. The supernatant, containing DMSO, hetastarch (if applicable), cell lysates, hemolysate (including free hemoglobin), and any chemokines and cytokines released up to that point, are removed and cellular sediment is resuspended in one volume of fresh isotonic infusion solution equal to or greater than the original product volume. For all products, this method achieves post-thaw stability in cases of prolonged thaw-to-infusion time and reduces the potential for infusion reactions, by significantly reducing the amount of DMSO, hetastarch (if applicable), cell lysates, hemolysate (including free hemoglobin), and any chemokines and cytokines. Moreover, according to Rubinstein et al. [13], this method averts post-thaw osmotic damage and stabilizes cell viability if the product is not infused immediately, and reportedly provided near total recovery of CFU progenitors. A number of recent studies have failed to confirm the latter observation as reviewed by Akel et al. [44]. The COBLT study reported no infusion-related toxicity without addressing the recovery controversy [32]. Laroche et al. [60] found 18% TNC loss with the dilution-wash method, with 11% loss due to the washing step alone. Other reports also show

significant loss of 10–25% for TNC, CD34+ and CFU [44–48]. Importantly, Regan et al. [48] showed that no-wash direct infusion provided significantly improved post-thaw TNC recovery (median 99.0%, mean 85.6%, $p < 0.01$) and viability (median 95.0%, mean 89.3%, $p < 0.01$) over post-thaw wash (TNC recovery median 78.6%, mean 77.4%, viability median 73.0%, mean 74.0%). This represents a significant 20% median TNC loss and 22% median viability reduction for post-thaw wash. Recently, even the NYBC in their HEMACORD product insert recommended a second centrifugation step for the supernatant to harvest some of the lost cells, which is a revision of the original NYBC wash method [13]. Both COBLT and NYBC recommends a final wash dilution ratio at 1:7.

Table 8 summarizes a comparison study comparing MaxCell CB products thawed and infused with bedside thaw versus post-thaw wash and showed improvement in 1-year overall survival, disease-free survival, transplant-related mortality, neutrophil, and platelet engraftment. Interestingly, limited cGvHD was higher while extensive cGvHD was lower for patients infused with unwashed MaxCell CB.

4.3. Reconstitution/dilution and no-wash method

The St. Louis CB Bank described a basic dilution and no-wash strategy [48]. Reconstitution with 1:1 ratio and ≥1:2 ratio of dextran-human serum albumin stabilized the hetastarch-processed RCR CB and PrepaCyte-CB, respectively, decreased viability loss with prolonged thaw-to-infusion time (for up to 8 h for HES-RCR CB), limited wash-related cell loss, and reduced preparation time and complexity. Barker et al. used a 1:4 ratio dilution and no-wash procedure for 104 RCR and 3 MaxCell products without severe AE [9]. However, the St. Louis group's own data [48] showed that no-wash direct infusion provided significantly better post-thaw TNC recovery (median 99.0%, mean 85.6%, $p < 0.01$) and viability (median 95.0%, mean 89.3%, $p < 0.01$) over no-wash dilution (TNC recovery median 78.0%, mean 78.4%, viability median 88.0%, mean 84.8%). This represents a substantial 21% median TNC loss and 7% median viability reduction for reconstitution compared to direct infusion.

Most CB banks now agree on the reconstitution, dilution, and washing solution composition of one volume of 25% human serum albumin (HSA) mixed with five volumes of 10% low-molecular-weight dextran 40), though the degree of CB product dilution varies between 2- and 16-fold. It should be noted that post-thaw washing or reconstitution can further reduce cell dose and viability as shown above [48], and is recommended for RCR CB by some CB banks, but not recommended by the manufacturer for MaxCell CB except in special circumstances [18, 21]. Regan et al. [48] showed a greater reduction of viability, TNC, and CD34 for unwashed and undiluted CB after 2 h *in vitro*. However, in clinical practice, direct infusion performed with bedside thaw should always be performed within 10–20 min to avoid DMSO toxicity (**Table 6**) and is almost never performed after such a prolonged post-thaw interval, making such *in vitro* comparisons clinically irrelevant except in cases where CB product manufacturer's recommendation is not followed. Importantly, the manufacturer of MaxCell CB products recommends completion of infusion of MaxCell CB products using the bedside thaw method within 10 min of initiation of thawing for adults at 5–10 mL/min for a 75-mL product. Several other groups have demonstrated that direct infusion without washing produces excellent

results for CB thawing [44, 47, 48, 60–68]. Chow et al. [18, 21, 50–59] was the first group to report superior clinical outcome with bedside direct infusion over post-thaw wash, with improved neutrophil and platelet engraftment, reduced transplant-related mortality, decreased extensive cGvHD, increased limited cGvHD, and enhanced overall and disease-free survival.

To address the question of whether the presence of residual RBC in the cryopreserved MaxCell products may adversely affect the outcome of HSCT, we have previously reported on the outcomes of 118 MaxCell CB transplants for patients with both hematological malignancies and nonmalignant indications [8]. Our experience indicates a 90.3% cumulative incidence for neutrophil (ANC500) engraftment, 75.5% for platelet 20K engraftment, 16.3% for 100-day transplant-related mortality, 65.5% for 1-year overall survival, and 51.6% for 1-year disease-free survival. This was followed by another series with 120 patients with nonmalignant indications [39] with similar outcome. At this point, after thousands of MaxCell CB products have been transplanted at around 300 transplant centers, that favorable experience reported previously has been maintained [[18, 35–43, 50–59], Chow et al. unpublished observations].

5. Conclusion

Because of the two- to threefold increased availability of high-cell dose CB products as a result of MaxCell CB processing, and the outstanding clinical outcome observed to date, it may be appropriate to ask whether it is reasonable to discard all the stem cell, progenitor cell, nucleated cell, and mononuclear cell in order to preserve storage space and reduce cost [13]. In fact, even the space cost savings argument is probably negated by the increased potential revenue generated from much higher availability of high-cell dose CB products. However, more importantly, is whether depriving patients of the opportunity to access the same HLA-matched CB products with higher cell doses can be justified in the name of economics. Cost, the original reason NYBC developed these RCR techniques, appears to be insufficient [13] if cell dose and in turn, clinical outcome and patient survival are compromised. As an example, a 25% improvement in infused cell dose can take a product from a suboptimal 2.0×10^7 TNC/kg body weight to an adequate 2.5×10^7 TNC/kg body weight.

To summarize, The results of outcome of the patients in the first MaxCell series [18] appear to be at least comparable to those reported in the medical literature [19, 23–34] and in some instances, superior to those reported for RCR CB products [18, 35–43, 50–59]. Though, there are no published data indicating inferior outcomes with transplantation using MaxCell units [8, 35–43, 50–59], such retrospective comparisons cannot be definitive. To analyze rigorously the outcomes of MaxCell CBT in comparison with RCR units, matched-pair comparisons for pediatric hematologic malignancies and thalassemia have shown significant improvements in overall survival, disease-free survival, transplanted-related mortality, and platelet engraftment for MaxCell CB products [35–38]. Moreover, when MaxCell CB products are coupled with direct infusion, significantly improved overall survival, disease-free survival, transplanted-related mortality, neutrophil, and platelet engraftment, higher limited cGvHD but lower

extensive cGvHD have been reported and subsequently confirmed in matched-pair comparisons. In conclusion, CB transplants using products processed by MaxCell CB processing technologies provide clinical outcome results that appear superior to results reported with the use of 1st Gen RCR units. When combined with bedside thaw techniques in most situations, further improvements can be expected [56–59].

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References

[1] Wagner J., Laughlin M., Petz, L. Summary of the 7th annual international cord blood transplantation symposium. *Biol. Blood Marrow Transplant*. 16, 12–27 (2010).

[2] Barker J.N., et al. Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow. *Biol. Blood Marrow Transplant*. 8, 257–60 (2002).

[3] Rocha V., Sanz G., Gluckman E. Umbilical cord blood transplantation. *Curr. Opin. Hematol*. 11, 375–85 (2004).

[4] Barker J.N., et al. Transplantation of two partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 105, 1343–7 (2005).

[5] Ballen K.K., et al. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol. Blood Marrow Transplant*. 13, 82–9 (2007).

[6] Jaing T.H., et al. Transplantation of unrelated donor umbilical cord blood utilizing double-unit grafts for five teenagers with transfusion-dependent thalassemia. *Bone Marrow Transplant*. 40, 307–11 (2007).

[7] Fernandez M.N., et al. Unrelated umbilical cord blood transplants in adults: early recovery of neutrophils by supportive co-transplantation of a low number of highly

purified peripheral blood CD34 cells from an HLA-haploidentical donor. *Exp. Hematol*. 31, 535–44 (2003).

[8] Chow R., et al. Analysis of hematopoietic cell transplants using plasma-depleted cord blood products that are not red blood cell reduced. *Biol. Blood Marrow Transplant*. 13, 1346–57 (2007).

[9] Barker J.N., et al. A "no-wash" albumin- dextran dilution strategy for cord blood unit thaw: high rate of engraftment and a low incidence of serious infusion reactions. *Biol. Blood Marrow Transplant*. 15, 1596–602 (2009).

[10] Takahashi T.A., et al. Multi-laboratory evaluation of procedures for reducing the volume of cord blood: influence on cell recoveries. *Cytotherapy* 8, 254–64 (2006).

[11] Regidor C., et al. Umbilical cord blood banking for unrelated transplantation: evaluation of cell separation and storage methods. *Exp. Hematol*. 27, 380–5 (1999).

[12] Alonso J.M. III, A simple and reliable procedure for cord blood banking, processing, and freezing: St Louis and Ohio Cord Blood Bank experiences. *Cytotherapy* 3, 429–33 (2001).

[13] Rubinstein P., Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc. Natl. Acad. Sci. U.S.A*. 92, 10119–22 (1995).

[14] Lapierre V., Cord blood volume reduction using an automated system (Sepax) versus a semi-automated system (Optipress II) and a manual method (hydroxyethyl starch sedimentation) for routine cord blood banking: a comparative study. *Cytotherapy* 9:165–9 (2007).

[15] Dazey B., et al. Cord blood processing by using a standard manual technique and automated closed system "Sepax" (Kit CS-530). *Stem Cells Dev*. 14, 6–10 (2005).

[16] Solves P., Planelles D., Mirabet V., Blanquer A., Carbonell-Uberos F. Qualitative and quantitative cell recovery in umbilical cord blood processed by two automated devices in routine cord blood banking: a comparative study *Blood Transfus*. 11, 405–11 (2013).

[17] Basford C., et al. Umbilical cord blood processing using Prepacyte-CB increases haematopoietic progenitor cell availability over conventional Hetastarch separation. *Cell Prolif*. 42, 751–61 (2009).

[18] Chow R., et al. Cell recovery comparison between plasma depletion/reduction and red cell reduction processing of umbilical cord blood. *Cytotherapy* 13, 1105–19 (2011).

[19] Rubinstein P., et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N. Engl. J. Med*. 339, 1565–77 (1998).

[20] Barker J.N., Byam C., Scaradavou A. How we search: a guide to the selection and acquisition of unrelated cord blood grafts. *Blood* 117, 2332–9 (2010).

[21] Petz L., Chow R. Letter to editor. *Blood* 118, 478–9 (2011).

[22] Page K.M., et al. Total colony-forming units are a strong independent predictor of neutrophil and platelet engraftment after unrelated cord blood transplantation: a single-center analysis of 435 cord blood transplants. *Biol. Blood Marrow Transplant*. 17, 1362–74 (2011).

[23] Migliaccio A.R., et al. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity *Blood* 96, 2717–22 (2000).

[24] Laughlin M.J., et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N. Engl. J. Med*. 351, 2265–75 (2004).

[25] Rocha V., et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N. Engl. J. Med*. 351, 2276–85 (2004).

[26] Davey S., et al. The London Cord Blood Bank: analysis of banking and transplantation outcome. *Br. J. Hematol*. 125, 358–65 (2004).

[27] Lapierre V., et al. The French Cord Blood Network: analysis of banking and transplantation outcome. *Transfusion*. 45, 59A (2005).

[28] Koegler G., Meyer A., Korschgen L., Platz A., Wernet P. *NMDP Council Meeting Abstract Book*. Abstract 2 (2005).

[29] Kernan N., et al. Umbilical cord blood transplantation in pediatric patients: results of the Prospective Multi-Institutional Cord Blood Transplantation Study (COBLT). *Biol Blood Marrow Transplant*. 12(Suppl. 1), 14 (2006).

[30] Ballen K., et al. Outcomes of 122 diverse adult and pediatric cord blood transplant recipients from a large cord blood bank. *Transfusion* 46, 2063–70 (2006).

[31] Eapen M., et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukemias: a comparison study. *Lancet* 369, 1947–54 (2007).

[32] Kurtzberg J., et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. *Blood* 112, 4318–27 (2008).

[33] Barker J.N., Scaradavou A., Stevens C.E. Combined effect of total nucleated cell dose and HLA match on transplanta- tion outcome in 1061 cord blood recipients with hematologic malignancies. *Blood* 115, 1843–9 (2010).

[34] Ballen K., et al. Effect of Cord Blood Processing on Transplant Outcomes after Single Myeloablative Umbilical Cord Blood Transplantation *Biol. Blood Marrow Transplant*. 21, 688–95 (2015).

[35] Graham M., et al. A retrospective study and matched pair analysis of 240 pediatric patients with Malignancies Transplanted with Plasma Depleted (PD) or Red Cell Reduced (RCR) Cord Blood (CB) Products. *Biol. Blood Marrow Transplant*. 14, 2S (2008).

[36] Jaing T., et al. Matched Pair Comparisons of Unrelated Cord Blood Transplantation (CBT) using Plasma Depleted Cord Blood Products (PD CB) Versus Red Cell Reduced (RCR) CB in 30 Pair of Patients with Thalassemia. *Cytotherapy* 10, S186 (2008).

[37] Chow R., et al. Matched pair comparisons of unrelated cord blood transplantation (CBT) using plasma depleted cord blood (PD CB) products versus red cell reduced (RCR) CB in 92 pair of patients with pediatric malignancies *Cytotherapy* 10, 1S 178 (2008).

[38] Chow R., et al. Matched pair comparisons of unrelated cord blood transplantation (CBT) using plasma depleted (PD) versus red cell reduced (RCR) cord blood (CB) products in 224 patients. *Transfusion* 48(2S), 134A, SP287 (2008).

[39] Petz L., et al. Analysis of 120 pediatric patients with non-malignant disorders transplanted using unrelated plasma depleted/reduced cord blood. *Transfusion* 52, 1311–1320 (2012).

[40] Chow R., et al. Unrelated cord blood transplantation (CBT) of 101 hemoglobinopathy (HGB) patients. *Biol. Blood Marrow Transplant*. 18(2–2), S268 #175 (2012).

[41] Chow R., et al. Cord blood (CB) APGAR score may be predictive of transplant related mortality (TRM), overall survival (OS) and disease-free survival (DFS) for plasma depleted/reduced CB products. *Biol.Blood & Marrow Transplant*. 17, 2–2 (2011).

[42] Nademanee A., et al. A retrospective audited analysis of 107 adult patients with malignancies transplanted with unrelated plasma depleted cord blood (PD CB)" *Biol. Blood Marrow Transplant*. 14(2S), 58 (2008).

[43] Rosenthal J., et al. Improved outcome for transplantation of pediatric patients with non-maligant disorders with unwashed plasma depleted cord blood (PD CB) *Biol. Blood Marrow Transplant*. 14 (2S), 28 (2008).

[44] Akel S., Regan D., Donna Wall D., Petz L., McCullough J. Current thawing and infusion practice of cryopreserved cord blood: the impact on graft quality, recipient safety, and transplantation outcomes. *Transfusion* 54, 2997–3009 (2014).

[45] Screnci M., Salvatori S., Carmini D., Arcese W. Does the volume reduction manipulation before cryopreservation influence cord blood cell recovery pretransplant? *Transfusion Med*. 17, 208–9. 41 (2007).

[46] Rodríguez L., et al. Washing of cord blood grafts after thawing: high cell recovery using an automated and closed system. *Vox Sang* 87, 165–72 (2004).

[47] Dornsife R., et al. Clinical cord blood units thawed with automated SEPAX cell processing system provides high quality post-thaw recoveries and viability from cryopreserved units. *Cytotherapy* 14(S), 221 (2012).

[48] Regan D.M., Wofford J.D., Wall D.A. Comparison of cord blood thawing methods on cell recovery, potency, and infusion. *Transfusion* 50, 2670–5 (2010).

[49] Petz L. Immunohematologic problems associated with bone marrow transplantation. *Transfusion Med. Rev*. 1, 85–100 (1987).

[50] Chow R., et al. Post-thaw washing prior to transplantation of umbilical cord blood units (UCB) that were depleted of plasma but not of red blood cells. *Biol. Blood Marrow Transplant. 12*(S1), 103 (2006).

[51] Chow R., et al. Hematopoietic stem cell transplantation (HSCT) using plasma depleted umbilical cord blood units (UCB) and the effect of post-thaw washing. In Wagner, J, Champlin, R, Petz, L. "Proceedings of the 4th Annual International Cord Blood Transplantation Symposium" *Biol. Blood Marrow Transplant*. 12, 1206–17 (2006).

[52] Chow R., et al. Post-thaw washing prior to transplantation of umbilical cord blood depleted of Plasma but not of red blood cells. *Transfusion* 46(9S), 4–5A (2006)

[53] Chow R., et al. Post-thaw washing prior to transplantation of umbilical cord blood (UCB) that were depleted of plasma but not of red blood cells. *Blood* 108, 398b (2006)

[54] Chow R., et al. Selection of post-thaw manipulations prior to transplantation of plasma depleted umbilical cord blood (PD CB) products. *Transfusion* 47(3S), 28A, S72–040A (2007)

[55] Chow R., et al. Avoidance of post-thaw wash prior to transplantation of plasma depleted cord blood (PD CB) is associated with improved engraftment & decreased severity of chronic GVHD (cGvHD) without increased relapse. *Blood* 110: 494a (2007)

[56] Chow R., et al. Negative impact of post-thaw washing on the overall survival (OS) and disease free survival (DFS) of patients receiving plasma depleted (PD) cord blood (CB) transplantation. *Biol. Blood Marrow Transplant*. 15, 2–2 (2009).

[57] Chow R., et al. Novel method to reduce extensive chronic GVHD (CGVHD) without increasing relapse for plasma depleted cord blood transplant (PD CBT). *Cytotherapy* 10(S1), 243 (2008).

[58] Chow R., et al. Avoidance of post-thaw washing prior to transplantation of plasma depleted umbilical cord blood (PD CB) improves outcome in a matched pair audited analysis of 258 patients. *Transfusion* 48(2S), 17A (2008).

[59] Chow R., et al. A novel method to reduce rates of extensive chronic GVHD (cGvHD) without increased relapse for cord blood transplant. *Biol. Blood Marrow Transplant*. 14(2S),11 (2008).

[60] Laroche V., et al. Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples. *Transfusion* 45, 1909–16 (2005).

[61] Antonenas V., Shaw P., Bradstock K.F. Infusion of unwashed umbilical cord blood stem cells after thawing for allogeneic transplantation. *Bone Marrow Transplant*. 34, 739 (2004).

[62] Patrick S., et al. Successful umbilical cord blood transplants in adults who received a nucleated cell dose 1×10^7 cells/kg processed by a post-thaw non-wash procedure. *Blood* 106, 580a (2005).

[63] Nagamura-Inoue T., et al. Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank. *Transfusion* 43, 1285–95 (2003).

[64] Sauer-Heilborn A., Kadidlo D., McCullough J. Patient care during infusion of hematopoietic progenitor cells. *Transfusion* 44, 907–16 (2004).

[65] McCullough J., McKenna D., Kadidlo D., et al. Issues in the quality of umbilical cord blood stem cells for transplantation. *Transfusion* 45, 832–41 (2005).

[66] Rowley S.D. Hematopoietic stem cell cryopreservation. In: Thomas, ED, Blume, KG, Forman, SJ, editors. Hematopoietic Cell Transplantation. Malden (MA): Blackwell Science; 481–92 (1998).

[67] Rowley S.D. Techniques of bone marrow and stem cell cryopreservation and storage. In: Sacher R.A., AuBuchon J.P., editors. *Marrow Transplantation: Practical and Technical Aspects of Stem Cell Reconstitution*. Bethesda (MD): American Association of Blood Banks; 105–27 (1992).

[68] Hahn T., et al. Use of non volume-reduced (unmanipulated after thawing) umbilical cord blood stem cells for allogeneic transplantation results in safe engraftment. *Bone Marrow Transplant*. 32, 145–50 (2003).