Clinical Evaluation of an Alternative Cord Blood Processing Method

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Abstract

In November of 2009, the St. Louis Cord Blood Bank (SLCBB) adopted the PrepaCyte-CB® method for the manufacture of cord blood units (CBU). This decision was made following an evaluation of alternative processing technologies, including automated methods, against the SLCBB's historical hetastarch method. The results yielded a trend toward increased post thaw TNC and CFU recoveries with the PrepaCyte-CB® method.

In the 17 months that have elapsed since the adoption of the PrepaCyte-CB® method, post processing product characteristics and potency measures have been consistently acceptable. Furthermore, the decrease in hematocrit of the final product has been remarkable (43.7% to 8.2%) while maintaining TNC recovery. To finalize the evaluation of this new processing reagent, the SLCBB has been compiling data for PrepaCyte-CB® units used in patient transplant.

A retrospective data analysis was performed to evaluate product safety and potency. Patient outcomes were provided either directly from the transplant center or through the Center for International Blood and Marrow Transplant Research (CIBMTR). Endpoints include probability of and time to neutrophil recovery and adverse events associated with product infusion.

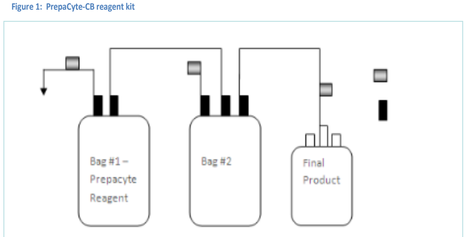
To date, 33 PrepaCyte-CB® CBUs have been shipped by the SLCBB for use in human transplantation. Outcomes data is available for 9 (27.3%) of these transplants. For the units that can be evaluated, all patients (n=9) have achieved neutrophil recovery with a mean time to recovery of 14.2 days. This is an impressive mark for cord blood and is significantly lower than the bank's historical experience of 22.4 days (P<0.01). Additionally, there have been no infusion related adverse events reported for the patient population.

While results are preliminary, they are encouraging for the use of this reagent in the manufacture of umbilical cord blood products for use in transplantation.

Background

The SLCBB has processed more than 5,000 cord blood units using the PrepaCyte-CB® reagent kit which is a set of three interconnected bags used to isolate white blood cells (Figure 1). The principle component of this methodology is the pre-measured PrepaCyte-CB® reagent (150mL) contained in bag one which, when mixed with umbilical cord blood (UCB), negates the zeta potential of the red blood cells forcing them to stack densely together. After sedimentation, WBC rich plasma is expressed into the next bag within the kit. Under centrifugation, the nucleated blood cells are forced out of the solution and cell-depleted plasma is expressed off as a waste product. The nucleated cells are re-suspended and transferred into the freezing bag component of the kit which is then ready for cryopreservation.

Figure 1: PrepaCyte-CB reagent kit



Background (continued)

Following an initial evaluation of processing methodologies, the SLCBB performed a more comprehensive analysis of the PrepaCyte-CB® reagent. Results from the trial have supported what was initially seen in the evaluation test group data. While post-processing TNC recoveries are consistent between PrepaCyte-CB® (86%) and the SLCBB Hespan (HES) method (85%), post-processing WBC recovery has increased from 87% with the SLCBB HES method to 91% with PrepaCyte-CB®.

A thaw control group has been established for PrepaCyte-CB®, for comparative purposes moving forward. Within this group (N=25), mean recoveries are reported as follows:
TNC = 89% (SD = 4%); TMNC = 87% (SD = 6%); CD34+ = 63% (SD = 15%);
CFU = 72% (SD = 14%); TB viability = 71% (SD = 6%).

One parameter of significance between PrepaCyte-CB® and all other methodologies initially evaluated, is hematocrit. PrepaCyte-CB® is extremely effective at depleting the RBC fraction at processing, leading to less RBC contamination post-thaw. Thaw control group data is reported in Table 1.

Table 1: Hematocrit Comparison (SLCBB Hespan vs. PrepaCyte-CB)

Parameter	Average Hct (%)		Min. Hct (%)		Max. Hct (%)	
	PrepaCyte	Hespan	PrepaCyte	Hespan	PrepaCyte	Hespan
Pre-Processing	35.5	35.2	28.3	26.6	48.4	44.3
Post – Processing	8.2	43.7	2.3	36.4	14.7	49.9
Post - Thaw	3.9	20.6	2.0	13.0	6.7	25.2

Materials & Methods

- A retrospective analysis was performed to evaluate cord blood processing method and impact on patient outcomes post infusion
- Outcomes data used for this analysis was provided through one of two methods:
  - Direct report to the SLCBB via the transplant center
  - Through the Center for International Blood and Marrow Transplant Research (CIBMTR)
- Patients for whom engraftment data was not available were excluded from this analysis
- Study populations were divided based on product processing method
- Study endpoints included probability of and time to neutrophil recovery and adverse events associated with product infusion

Results

- Cord blood units processed with PrepaCyte-CB® have a higher mean total nucleated cell count (TNC), TNC percent recovery, CD34+ count, and colony forming cell count when compared to products processed with Hespan (Table 2).
- Comparison of post thaw TNC percent recovery (as reported by transplant centers) was significantly higher for PrepaCyte-CB® products (86.2%) as compared to Hespan products (81.5%) p<0.05.
- There was not a significant difference between the methods when comparing post thaw percent viability p>0.05.
- When evaluating probability of and time to patient neutrophil recovery, there is a significant difference between the groups with respect to median time to recovery. The PrepaCyte-CB® cohort had a median time of 15 days as compared to 21 days for the Hespan group. Note: Variables such as conditioning regimen, patient age, disease, and cell dose have been controlled for to the extent possible given the limited sample size for the PrepaCyte-CB® group.

Table 2: Comparison of Post Processing Counts

Measure	Hetastarch (Hespan)	PrepaCyte® -CB
Post Processing TNC		
N	20,787	5,134
Mean	1183.0	1386.3
Post Processing TNC Recovery		
N	20,827	5,134
Mean	83.4	84.0
Post Processing CD34+		
N	20,834	5,134
Mean	5.1	5.8
Post Processing CFU		
N	17,910	5,134
Mean	11.3	19.5
Post Processing Viability		
N	20,420	5,134
Mean	94.9	93.3

Table 3: Comparison of Post Thaw Counts

Measure	Hetastarch (Hespan)	PrepaCyte® -CB
Post Thaw TNC		
N	1066	38
Mean	1389.6	1992.2
Post Thaw TNC Recovery		
N	1061	38
Mean	81.5	86.1
Post Thaw Viability		
N	997	31
Mean	78.0	74.3

Results (continued)

Figure 2: Mean Post Thaw Percent TNC Recovery and Percent Viability

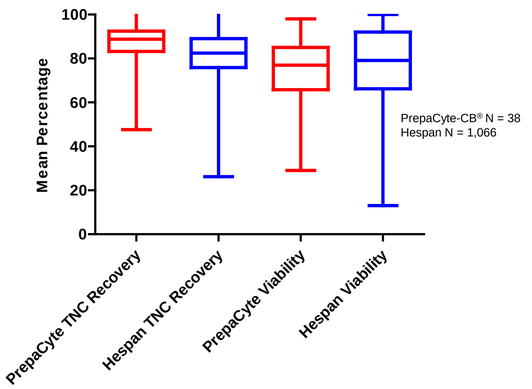
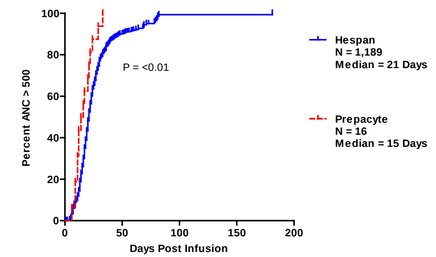


Figure 3: ANC Recovery by Processing Method



Conclusions

Laboratory evidence has previously shown PrepaCyte-CB® to be effective in recovering the targeted WBC population while reducing incident red blood cells during cord blood processing. Most compelling about the performance of this reagent is its ability to retain a high level of potency post thaw as demonstrated by superior recovery of colony forming units. This observation appears to translate to significantly improved engraftment times (15 days from the benchmark of 21 days), potentially reducing length of stay and morbidity/mortality associated with transplantation events.

The St. Louis Cord Blood Bank collects, processes, and distributes high-quality cord blood products for cellular therapy applications, focusing on the development of effective cures through advancing the science of characterization, manufacturing, and translational research practices in preparation for transplantation and regenerative medicine therapies. Since 1996, the SLCBB has received over 100,000 donations through our community of altruistic families and medical professionals. From an inventory of 24,000 products, over 1,950 have been distributed for life saving therapies.

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