Clinical Outcome of Unrelated Cord Blood Transplants: An Analysis of Processing Method and Freezer Storage on Transplants from New York Blood Center National Cord Blood Program

Background: The New York Blood Center (NYBC) established the first public cord blood bank, now known as the NYBC National Cord Blood Program (NCBP), in 1992, collected its first donated cord blood unit (CBU) in February 1993 and provided the first unrelated cord blood graft in August 1993. To date, the NCBP has collected 38,718 units and has provided CBUs to 2,326 patients around the world.

Four different methods used to process, freeze and store CBUs since NCBP inception. Initially, CBUs were cryopreserved as whole blood with an equal volume of 20% DMSO, placed in standard freezing bags (Baxter) and aluminum canisters, frozen in -80° C freezers and stored in racks of 5 units under liquid nitrogen in conventional Dewars (**method = "whole blood-conventional Dewar"**). In 1994, we developed a method to reduce the frozen volume, adding Hespan (hetastarch) followed by centrifugation and manual removal of excess red blood cells and plasma to achieve a uniform final volume of 20 mL. All CBUs collected since October 3, 1994 have been volume reduced (VR) and cryoprotected with 5 mL of 50% DMSO (10% final concentration). Initially, VR units were placed in standard freezing bags (Baxter) and aluminum canisters, frozen in -80° C freezers and stored in racks of 5 units under liquid nitrogen in conventional Dewars (**method = "volume reduced-conventional Dewar"**). Manual transfer of units from the -80° C freezer to the conventional Dewar and, subsequently, for retrieval prior to shipment to transplant centers requires removal of the entire racks. Thus, these units may be exposed to ambient air resulting in multiple transient warming events (TWEs). Since May 4, 1999, all VR units were placed in specially designed freezing bags (Pall), frozen and stored in canisters at individual addresses in the ThermoGenesis BioArchive™ Symtem freezers. The BioArchive System performs the initial controlled rate freezing in the gas phase above liquid nitrogen, records the freezing curves and robotically transfers the unit to its assigned storage address under liquid nitrogen within the same Dewar (**method = "volume reduced-BioArchive"**). When units are retrieved for shipment, the BioArchive removes the individual unit robotically, transfers the unit from liquid nitrogen into insulated sleeves in the gas phase. The insulated unit is then transferred to a liquid nitrogen cooled dry shipper for shipment to the transplant center. Thus, once frozen, BioArchive units are not exposed to ambient temperature and, thus, are not exposed to TWEs. Beginning on August 2, 2007, manual processing was replaced by automated processing using the ThermoGenesis AutoExpress™ (AXP) in a closed system without HES. Tesulting VR CBUs were cryoprotected and frozen as before in the BioArchive (**method = "AXP-BioArchive"**). The cumulative number of CBUs processed and frozen to date by each method are:

Whole Blood-Conventional Dewar	2,202
Volume Reduced-Conventional Dewar	5,313
Volume Reduced-BioArchive	28,227
AXP-BioArchive	2,974

As of February 28th, the NCBP had provided 2,541 cord blood grafts to 2,325 patients who were unrelated to the donor (some patients have had more than one transplant and others have gotten more than one CBU simultaneously). Units were shipped frozen and thawed at the Transplant Center just prior to transplantation. Most patients were transplanted in Transplant Centers in the United States (71%), although increasingly units have gone to non-US Centers (43% in 2006). Most patients have been children (68% < 16 years old), although the proportion of patients who are adults increased substantially after two 2004 New England Journal of Medicine papers on cord blood transplantation in adults to 47% in 2006. Most patients were transplanted for leukemia (62%) or genetic diseases (23%).

Transplant Centers report on the condition of the unit on arrival and after thawing (including post-thaw cell count and viability) and on the patient's clinical outcome at 3, 6 and 12 months post-transplant and annually thereafter. A recent collaborative study with the Center for International Bone Marrow Transplant Registry (CIBMTR), compared unrelated bone marrow and cord blood transplants in U.S. children with leukemia or myelodysplasia (in press, Lancet). The total nucleated cell (TNC) dose for cord blood grafts in this study was 5 to 10-fold lower than that of bone marrow and cord blood recipients had delayed myeloid and platelet engraftment compared to bone marrow recipients. Nevertheless, patients given cord blood with an adequate TNC dose ($\geq 3.0 \times 10^7$/kg) and an HLA-A, -B, -DRB1 match of 5/6 or better had a five-year survival that was as good as or better than that of patients given fully matched bone marrow transplants. Although not a randomized clinical trial, these data suggest that cord blood can have survivals as good as that of bone marrow from an unrelated donor. Indeed, clinical practice is already changing to reflect this conclusion. In fact, the annual survey of the World Marrow Donor Association (WMDA) shows that while transplantation with cord blood grafts continues to increase, that of bone marrow has declined suggesting that cord blood may now be being used in reference to bone marrow in some Centers.

<u>Analyses</u>: Outcome data has been reported on more than 90% of patients given NYBC CBUs for transplantation between 1993 and 2005. Reporting is not yet complete on more recent transplants. The analyses presented herein, therefore are limited to the 1993-2005 period. The first transplant with a CBU that had been frozen and stored in a BioArchive freezer took place in 1999. Because of the possibility of changes in clinical practice, cell dose of grafts used and the types of patients being transplanted, we included calendar year as a variable in multivariate analyses. The outcome endpoints of interest were engraftment (absolute neutrophil count $\geq 500/\mu L$ and platelet count $\geq 50{,}000/\mu L$), transplant

related mortality (non-relapse deaths) and overall mortality. Comparisons of the time of outcome events were performed through Kaplan-Meier estimates and, in multivariate analyses, by Cox regression. In these analyses, cell dose provided by the CBU was expressed as the cell dose (i.e., total nucleated cells or TNC) per kilogram of patient body weight at the time of transplant. Analyses of cell dose effects used either categorical groupings of post-processing cell dose per kg or cell dose as a log transformed continuous variable.

Clinical Outcome: Among the 2,325 patients who have received cord blood grafts from the NCBP, 2,067 had single-unit transplants, 254 have gotten more than one cord blood unit simultaneously (additional units either from our own or a different cord blood bank) and 4 got a CBU and a haplo-identical related bone marrow graft. The table below shows the number of cord blood units given as single-unit transplants by year and type of processing and freezer.

Transplant Year	Whole Blood- Conventional Dewar	Manual Volume Reduced Conventional Dewar	Manual Volume Reduced BioArchive	AXP BioArchive
1993	2	0	0	0
1994	15	0	0	0
1995	61	28	0	0
1996	104	105	0	0
1997	93	134	0	0
1998	92	143	0	0
1999	45	109	1	0
2000	20	77	14	0
2001	12	31	58	0
2002	6	32	90	0
2003	7	22	111	0
2004	3	14	133	0
2005	3	22	192	0
2006	2	19	221	0
2007*	0	6	39	1
TOTAL	465	742	859	1

* through 2/28//07

The table below shows the year that patients given single units were transplanted and the numbers that have reported post-transplant outcome data to date.

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		Conventional Dewar	BioArchive	BioArchive
1993	2	0	0	0
1994	15	0	0	0
1995	61	28	0	0
1996	104	105	0	0
1997	93	134	0	0
1998	92	143	0	0
1999	45	109	1	0
2000	20	77	14	0
2001	12	31	58	0
2002	6	32	90	0
2003	7	22	111	0
2004	3	14	133	0
2005	3	22	192	0
2006	2	19	221	0
2007*	0	6	39	1
TOTAL	465	742	859	1

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The table below shows the year that patients given single units were transplanted and the numbers that have reported post-transplant outcome data to date.

Transplant Year	Number Given Single-Unit Transplants	Number with Outcome Data Reported
1993	2	2
1994	15	15
1995	89	89
1996	209	209
1997	227	227
1998	235	234
1999	155	155
2000	111	113
2001	101	97
2002	128	121
2003	140	114
2004	150	119
2005	217	122
2006	242	7
2007*	46	0
TOTAL	2,067	1,622

* through 2/28/07

In the analyses that follow, transplant outcome will be restricted to transplants that took place from 1993 to 2005, to maximize the proportion with outcome data reported. Reports on outcome of patients transplanted in 2006-07 are too few, as yet, to permit unbiased assessment. The analysis, therefore, includes data on the 1,615 patients among the 1,779 transplanted between 1993 and 2005. Of these, 454 given cord blood units processed as whole blood and stored in conventional Dewars, 704 given cord blood that was volume reduced and stored in conventional Dewars and 457 given cord blood that was volume reduced and stored in BioArchive freezers (all transplanted since 1999). The analysis focuses on engraftment endpoints (myeloid and platelets) and overall patient survival, endpoints that are directly related to cell dose and are, thus, most relevant to the quality of processing and freezing. Analyses utilized Kaplan-Meier estimates of the incidence and timing of various endpoints in univariate analyses and Cox Regression for multivariate analyses.

Transplant centers reported on post-thaw TNC recovery for 1,365 patients and on post-thaw cell viability on 1,221. Post-thaw TNC recovery was significantly higher for BioArchive units than for units that were either non-reduced or volume reduced but stored in conventional Dewars (mean proportion of cells recovered ± standard error of the mean = 0.86 ± 0.02, 0.75 ± 0.01 and 0.70 ± 0.01, respectively. P value < 0.001 for each comparison). Similarly, the viability reported on cells recovered post-thaw was higher in BioArchive units than in volume reduced unit that were either non-reduced or volume reduced but stored in conventional Dewars (mean percent of viable cells ± standard error of the mean = 88.0% ± 0.8, 84.4% ± 0.8 and 82.0% ± 0.7 respectively. P value =

0.001 and <0.001 for BioArchive vs. the other two groups, respectively). Viability of whole blood units was also higher than that of volume reduced units from conventional Dewars (P = 0.03).

Data on time to absolute neutrophil count ≥ 500/μL (ANC 500) was available on 1,494 patients, including 384 whose graft had been frozen and stored in BioArchive freezers. Time to ANC 500 correlated with TNC dose per kilogram of patient body weight at the time of transplant, whether the Transplant Center was in the United States, with certain disease categories and with Methotrexate prophylaxis for graft vs. host disease (GvHD) and level of HLA match between the patient and the graft. Patients engrafted faster if they received grafts that provided a relatively high TNC dose, were transplanted in US centers, did not have severe aplastic anemia, Fanconi anemia or chronic myelogenous leukemia, were not given methotrexate post-transplant for GvHD prophylaxis and received a fully HLA-matched graft (6/6 antigens at the HLA-A,-B, -DR loci). Patients given volume reduced cord blood units that had been stored in conventional Dewars slower time to ANC 500 than those given units frozen as whole blood (see figure below), despite the fact that patients in the two groups received similar TNC doses in their grafts (geometric mean = 4.6 x 10^7/kg and 4.4 x 10^7/kg, respectively, p value = 0.3). Patients given volume-reduced units that were frozen and stored in BioArchive freezers received a higher TNC dose on average (geometric mean = 6.3 x 10^7/kg, p value compared to non-reduced CBU recipients < 0.001) and had myeloid engraftment that was faster than that those given either non-reduced or volume reduced units stored in conventional Dewars. In a multivariate analysis that took into account TNC dose, US center, methotrexate prophylaxis, HLA match and disease category and processing/freezer method, all variable remained significantly predictive of time to ANC 500 (Table 1). Most importantly, compared to patients given volume reduced units that were frozen and stored in conventional Dewars, patients given either whole blood units stored in conventional Dewars or volume reduced units frozen and stored in the BioArchive had significantly faster engraftment and higher relative risks (RR) for time to ANC 500. Engraftment rates for the later two groups of patients did not differ significantly from each other in a multivariate analysis with whole blood units in conventional Dewars as the reference (RR = 0.98, 95% confidence interval = 0.84-1.16, p value = 0.9).

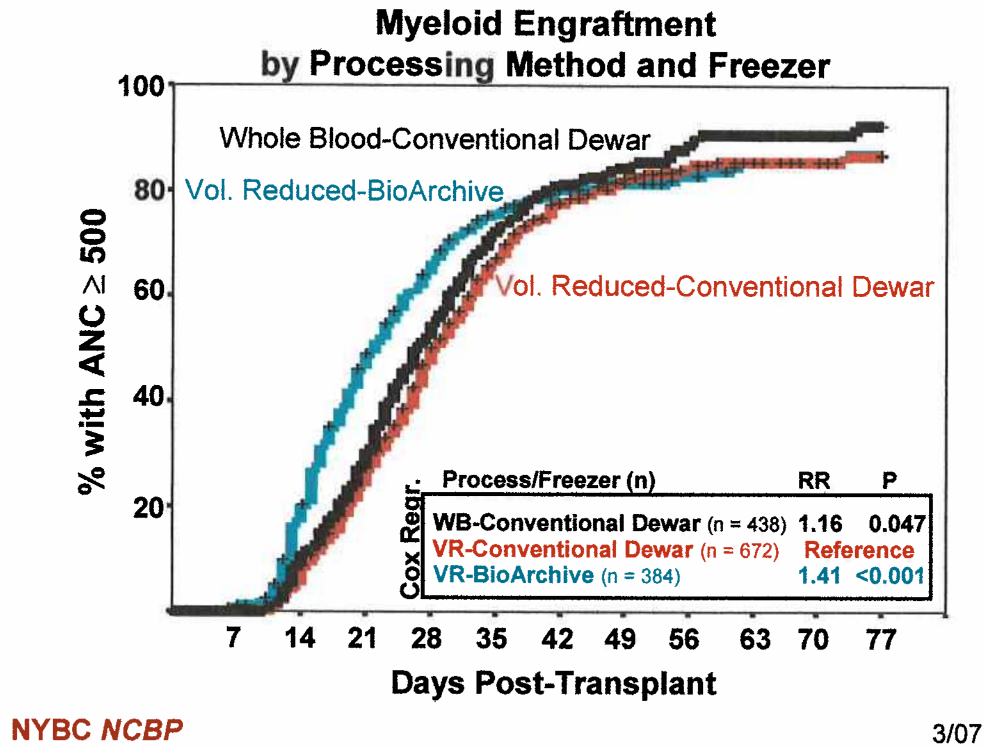


Table 1. Multivariate Analysis of Time to Myeloid Engraftment (ANC 500). 4 patients without high resolution DRB1 typing were excluded.

Variable	Number of Patients	Relative Risk (95% CI)	P Value
Processing-Freezer:			
WB-Dewar	434	1.25 (1.08 – 1.45)	0.002
VR-Dewar	672	Reference	
VR-BioArchive	384	1.25 (1.07 – 1.45)	0.004
TNC/Kg (natural log)	1490	1.53 (1.40 – 1.66)	< 0.001
HLA Mismatch (4 not determined):			
None	82	1.41 (1.08 – 1.85)	0.011
1	525	Reference	
2	787	0.97 (0.85 – 1.11)	0.7
3	96	0.81 (0.62 – 1.06)	0.13
Methotrexate GvHD Prophylaxis:			
Methotrexate	209	0.70 (0.59 – 0.84)	< 0.001
Unknown	294	0.63 (0.53 – 0.76)	< 0.001
Other	987	Reference	
Center Experience:			
< 25 Patients	620	0.86 (0.75-0.99)	0.034

Center:			
Non-US	336	0.74 (0.62 – 0.87)	< 0.001
US	1154	Reference	
Disease Category:			
Other	1274	Reference	
SAA, FA, CML	216	0.69 (0.57 – 0.84)	< 0.001

CI = Confidence Interval
VR = Volume Reduced. WB = Whole Blood.
SAA = severe aplastic anemia. FA = Fanconi anemia. CML = chronic myelogenous leukemia.

Data on time to a sustained platelet count of ≥ 50,000/μL is available of 1,434 patients, including 355 recipients of BioArchive CBUs. As with myeloid engraftment, compared to recipients of volume-reduced units stored in conventional Dewars, patients given volume reduced units that were frozen and stored in BioArchive freezers had faster platelet engraftment (see figure below). This relationship persisted in the multivariate analysis and retained statistical significance (Table 2). In the multivariate analysis for platelet engraftment, only US/non-US Transplant Center, TNC dose, HLA match and processing-freezing method were significant predictors of time to platelet 50,000. Speed of platelet engraftment was not significantly different between patient given whole blood units and those given BioArchive units (RR = 1.15 with 95% confidence interval = 0.93-1.41, p value = 0.2).

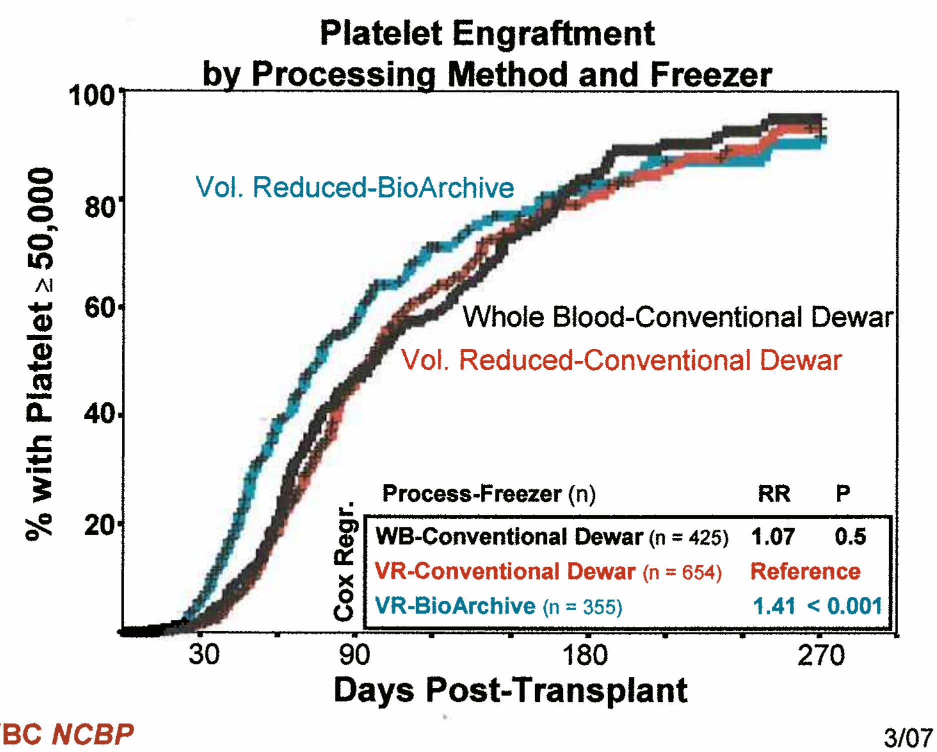
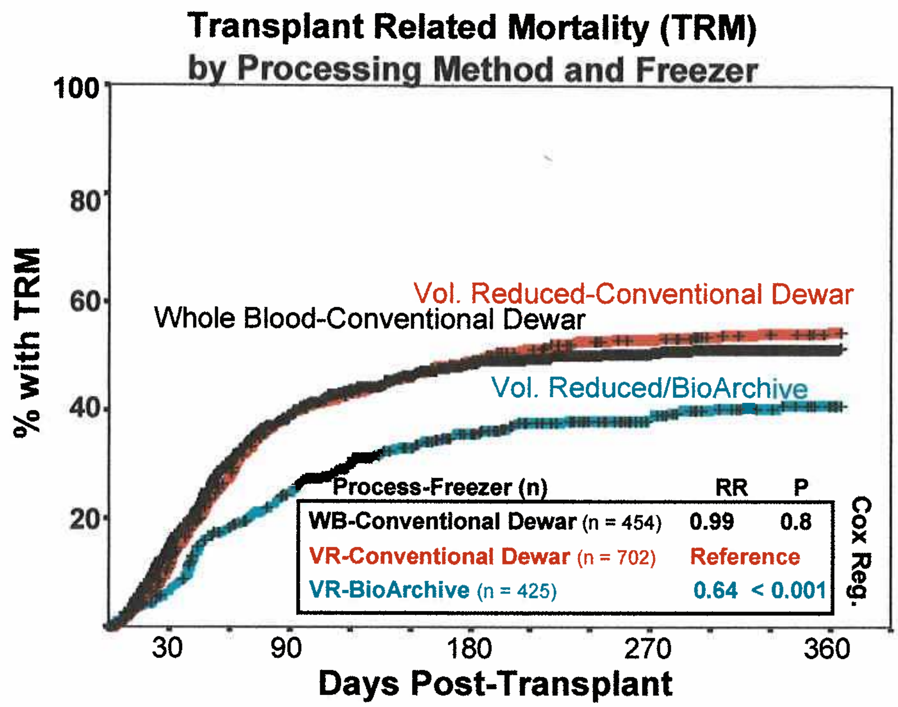


Table 2. Multivariate Analysis of Time to Platelet Engraftment (Platelet ≥50,000).

Variable	Number of Patients	Relative Risk (95% CI)	P Value
Processing/Freezer:			
WB/Dewar	421	1.14 (0.94 – 1.38)	0.19
VR/Dewar	654	Reference	
VR/BioArchive	355	1.30 (1.08 – 1.57)	0.006
TNC/Kg (natural log)	1430	1.53 (1.38 – 1.69)	< 0.001
Center:			
Non-US	322	0.81 (0.66 – 1.00)	0.053
US	1108	Reference	
HLA Mismatch (4 not determined):			
None	81	1.90 (1.42 – 2.55)	< 0.001
1	498	Reference	
2	760	0.92 (0.77 – 1.09)	0.3
3	91	0.73 (0.50 – 1.08)	0.11

Most deaths among hematopoietic transplant recipients occur in the first 6 months post-transplant. Transplant related mortality and overall mortality (inverse of survival) analyses, therefore, were limited to the first year post-transplant. In addition to variables associated with engraftment (TNC dose, HLA match level, center experience and disease category), TRM and survival were associated with the stage of disease at the time of transplant, patient age and ethnicity and the presence of antibody to cytomegalovirus (CMV) pre-transplantation. Patients who received a cord blood unit that had a 6/6 or 5/6 HLA-A, -B, -DRB1 match had lower post-transplant TRM and higher survival than those who received units that were matched for 4/6 antigens or less. Patients transplanted when they had advanced leukemia (in relapse or refractory to treatment for acute leukemia or in blast crisis for CML) had higher TRM and lower survival than patients with less advanced leukemia or other diseases. And patients who had been infected with CMV prior to their transplant (pre-transplant anti-CMV positive) were more likely to have a CMV infection post-transplant and had higher TRM and lower survival. Patients given units frozen and stored in BioArchive freezers have had lower TRM and higher overall survival than recipients of other units. In the multivariate analysis, receipt of a graft from the BioArchive remained predictive of better survival, when compared to recipients of volume reduced units stored in conventional Dewars as the reference group (see Figures below and Tables 3 and 4).



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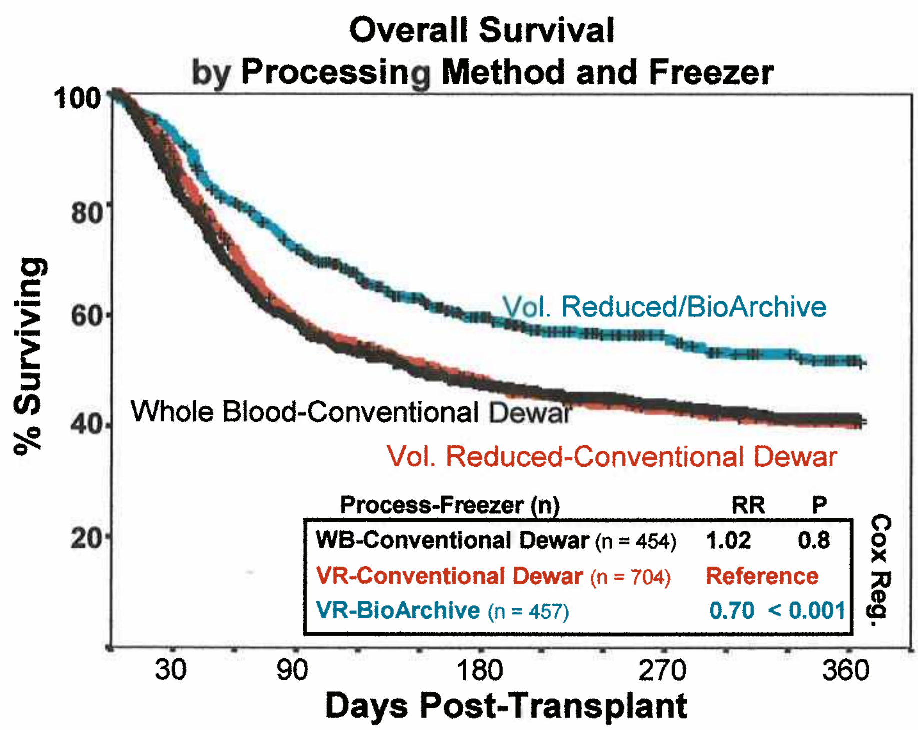

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Table 3. Multivariate Analysis of Transplant Related Mortality (TRM) and Overall Mortality During the First Year Post-Transplant.

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Transplant Related Mortality: (38 excluded because of missing data)

HLA Mismatch:			
0	87	0.6 (0.4-0.9)	0.021
1	560	Reference	
2	830	1.2 (1.1-1.5)	0.012
3	100	1.2 (0.9-1.6)	0.3
TNC Dose/Kg (natural log)	1577	0.78 (0.68-0.90)	< 0.001
Age (years):			
0-11	1050	Reference	
12-24	284	1.3 (1.1-1.7)	0.007
≥ 25	243	1.5 (1.2-1.9)	0.001
High Risk ALL, AML, CML	291	1.2 (0.98-1.4)	0.067
Non-Caucasian	702	1.3 (1.1-1.5)	0.004
Pre-Transplant CMV Antibody:			
Negative	690	Reference	
Positive	678	1.2 (1.03-1.4)	0.018
Unknown	196	1.2 (0.98-1.6)	0.067
Center Experience: < 25 Patients	678	1.2 (1.02-1.4)	0.030
Processing/Freeze Method:			
Whole Blood-Conv. Dewar	450	1.0 (0.8-1.2)	0.9
Vol. Red.-Conv. Dewar	702	Reference	
Vol. Red.-BioArchive	425	0.7 (0.6-0.8)	< 0.001

Overall Mortality: (4 excluded because of missing data)

HLA Mismatch:			
0	89	0.6 (0.4-0.9)	0.020
1	569	Reference	
2	852	1.2 (0.99-1.4)	0.052
3	101	1.1 (0.8-1.5)	0.5
TNC Dose/Kg (natural log)	1611	0.81 (0.71-0.91)	0.001
Age (years):			
0-11	1075	Reference	

High Risk ALL, AML, CML	291	1.5 (1.2-1.7)	< 0.001
Non-Caucasian	722	1.2 (1.1-1.4)	0.004
Pre-Transplant CMV Antibody:			
Negative	690	Reference	
Positive	691	1.2 (1.1-1.4)	0.006
Unknown	230	1.2 (0.9-1.5)	0.16
Center Experience: < 25 Patients	708	1.1 (0.96-1.3)	0.16
Processing/Freeze Method:			
Whole Blood-Conv. Dewar	450	1.04 (0.9-1.2)	0.6
Vol. Red.-Conv. Dewar	704	Reference	
Vol. Red.-BioArchive	457	0.7 (0.6-0.9)	< 0.001

Because BioArchive units have only been used relatively recently, we also looked at survival and year of transplant, comparing patients transplanted from 1993 to 1998 with those transplanted in 1999 or later (when BioArchive units began to be used). In this analysis, the year of transplantation did not associate with the probability of survival.

When recipients of CBUs cryopreserved as whole blood was used as the reference group in the multivariate analyses of survival TRM and overall mortality, recipients of BioArchive units continued to have a significantly better outcome (p value <0.001).

Conclusions: CBUs that were volume reduced and stored in conventional Dewars have had reduced myeloid engraftment compared to units stored as whole blood in conventional Dewars and volume reduced units stored in the BioArchive. This relationship is presumably related to improved post-thaw cell recovery and viability reported for BioArchive units and to probable protection against transient warming events (TWEs) that are expected to have the greatest effect on volume reduced CBUs stored in conventional Dewars due to their lower thermal mass and lack of protection from ambient temperatures during CBU removal from freezers. Significantly improved TRM and survival in recipients of BioArchive CBUs compared with recipients of units processed and frozen by other methods may be explained in part by improved speed of engraftment and persisted in multivariate analyses that took into account recognized risk factors for post-transplant survival.

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