

Placental Blood Program
New York Blood Center
310 East 67th Street

July 15, 2000

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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20837

Re: Docket Number 97N-0497

We are submitting the following Comments and Appendices in response to the Agency's request for comments proposing standards to ensure the safety and effectiveness of minimally manipulated hematopoietic stem/progenitor cells derived from placental/umbilical cord blood for use by unrelated recipients.

These Comments focus on areas that are felt to be most germane to the Agency's purpose and in which our group has developed experience over the last seven years, the last four under BB IND 6637. We also want to reiterate our invitation to the Agency to ask for factual data about any additional aspects of our experience in this area.

The data have been generated by the Placental Blood Program of the New York Blood Center. Briefly, this Program was organized as part of an effort to investigate the feasibility, safety, and possibly, the effectiveness of harvesting the neonatal blood left within the placental and umbilical cord vasculature (designated **placental** or **cord** blood and abbreviated PCB), as a source of hematopoietic stem and progenitor cell for transplantation to marrow-ablated allogeneic recipients. Until 1995, the Program was supported by an Award from the National Heart, Lung and Blood Institute and since 1996 it has generated its financial support from the collection of reimbursement fees determined by FDA pursuant to conditions set forth with respect to IND BB 6637.

The **Objectives** of this program are to

1. collect PCB from delivered placentas so as not to change in any meaningful way the management of Labor,
2. obtain Informed Consent from the mother of the PCB donor to test and use the PCB for transplantation, including obtaining a detailed interview with the mother with regard to the family's history of genetic and infectious disease and the mother's permission to review the mother and baby's clinical charts,
3. cryoprotect PCB and store it frozen at liquid nitrogen temperature (-196°C),
4. perform tests for infectious disease, type HLA and other blood group markers for histocompatibility matching to eventual recipients,
5. maintain samples both integral to (attached) to the container of the PCB unit or graft and detached from it and from the blood of the donor's mother for further testing,

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6. provide appropriately selected PCB grafts to specific recipients and obtain pertinent information on their disease and clinical course before and after transplantation
7. develop and maintain a database with specific factual information obtained from the mother's interview, all results from blood tests of the donor and mother and from the eventual recipient of the PCB as a transplant.

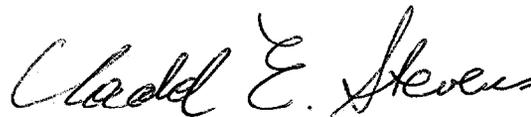
Pursuant to the IND in effect since June, 1996, the Program has kept detailed records of its activities, methods and results and has reported on these aspects. Together with clinical colleagues, we have also reported on the outcomes of PCB transplantation in scientific papers and meeting reports.

We have participated in the elaboration of the FAHCT/NETCORD'S "Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release", which have also been submitted to this Docket. Our present submission focuses on data from our Program which we believe is of importance to the drafting of effective regulation and we stand ready to submit additional data and analyses as needed. We also request that the Agency put into the Public Domain all data submitted with our Program's Annual Reports.

We begin with a description of some general aspects of the Program experience to date.



Pablo Rubinstein, M.D.
Director, Placental Blood Program

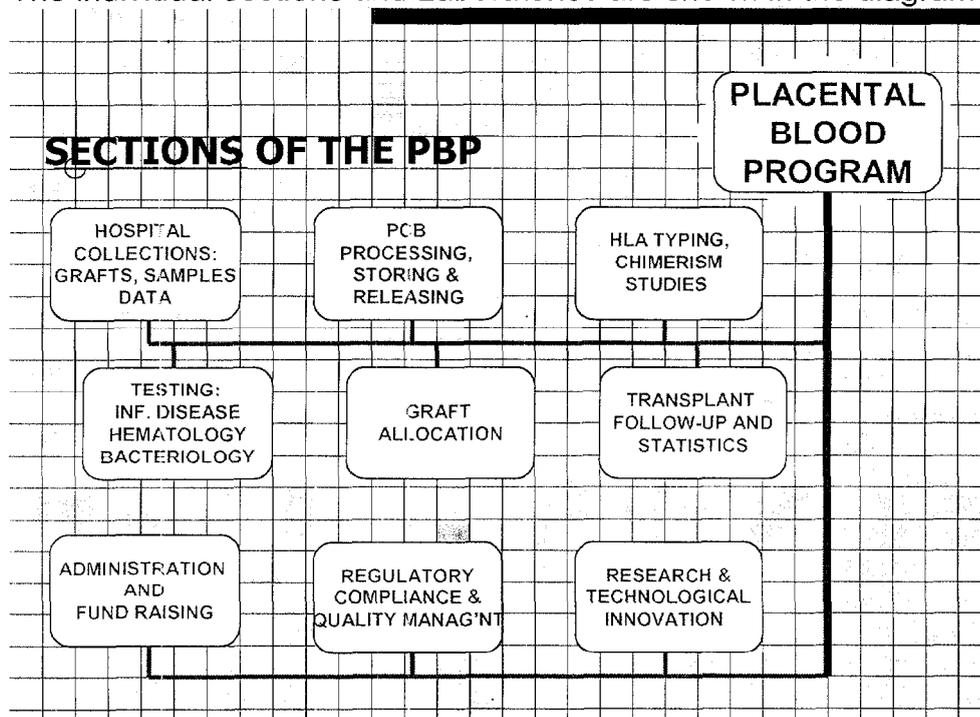


Cladd E. Stevens, M.D.
Medical Director, Placental Blood Program

A. Program Organization. The Placental Blood Program consists of the following modules:

1. *HOSPITALS:* collection of PCB, data and mother's blood samples.
2. *PROCESSING:* separation of test-samples, volume reduction and freezing of PCB. Testing for ABO, Rh and Hb. Shipping grafts to Patient's Transplant Center.
3. *HLA TYPING:* grafts, mothers, patients' pre- and post-transplant evaluation and follow-up.
4. *TESTING:* infectious disease, bacteriology, hematology, hematopoietic precursors.
5. *QUALITY MANAGEMENT:* SOP validation, documentation, licenses, accreditation. NETCORD-FAHCT.
6. *TRANSPLANT ALLOCATION:* clinician queries, arranging confirmatory tests, conflict resolution.
7. *TRANSPLANT FOLLOW-UP AND DATA ANALYSIS.*
8. *DATABASE AND COMPUTER APPLICATIONS:* search and control.

The individual sections and Laboratories are shown in the diagram below:



B. The findings reported in 1998 on the clinical outcomes of the first 562 patients transplanted with the Program's Units, has been confirmed and extended by our most recent analysis. The data are summarized below.

1. Program's highlights.

Date	Event
September 1992	Three-year Award from NHLBI announced.
February 1, 1993	First Collection of PCB for clinical use, at Mt Sinai Hospital
June 1993	First Search Requests for patients in need of marrow replacement received by the Program
August 1993	First unrelated PCB Transplant at Duke University
July 1994	Introduction of Dextran/Albumin post-thawing wash
October 1994	Pre-freezing Volume Reduction of PCB
September 1995	NHLBI's Award ends.
December 1995	Transplant No. 100 performed
June 1996	IND assigned No. BB 6637 by FDA.
February 1997	Collection facilities moved to Brooklyn Hospital
September 1998	a. Introduction of Closed Processing Bag Set and two-compartment Freezing Bag b. Introduction of Robotic LN2 Freezer
November 1998	Publication of NEJM Paper (562 consecutive patients.)
November 1999	a. Unit #1000 Shipped b. Second Collection Site at North Shore Hospital.

2. Summary of main clinical activities as of 6/30/2000

PCB Units In Storage:	10935
No. of Search Requests received:	9714
PCB Units Shipped:	1049 (in 132 Transplant Centers).
Total Patients Transplanted:	1019

3. Number of Patients Transplanted, by year.

Year	Number
1993	2
1994	15
1995	89
1996	209
1997	228
1998	236
1999	154
2000	86 (to 6/30/2000)

4. Patient's Age at Transplantation (8/24/1993—6/30/2000).

<u>Patient's Age (Years)</u>	<u>Number of patients</u>
<2	203
2-5	216
6-11	234
12-17	142
≥18	224

5. Ethnicity and Numbers of Transplanted Patients (8/24/1993—6/30/2000) and PCB Units (as of 7/12/2000)

	Txp. Patients		Units	
	Number	%	Number	%
Caucasoid	651	64.5	3821	37.5
African-American	135	13.4	2806	27.6
Hispanic	155	15.4	2361	23.2
Asian	37	3.7	387	3.8
Others	17	1.7	801	8.9
Native American	5	0.5	?	

6. Diagnosis of Transplanted Patients (8/24/1993—6/30/2000).

Leukemia	666
Lymphoma	26
Genetic Disease	242
Myelodysplasia	42
Severe Aplastic Anemia	31
Other conditions	12

7. Post-Transplant Events (933 Patients Transplanted through 12/99).

Suffered Autologous Reconstitution	23
Received a Back-up Graft	43
Relapsed	91
Died (all causes)	424

8. Variables that affect the speed of Engraftment (Reaching an absolute Neutrophil Count (ANC) = 500/ μ L). Multivariate Analysis by Cox Regression.

Significant Variable	p value *
Total Nucleated Cell Dose (TNC)	< 0.001
HLA Match Grade	0.005
Disease Diagnosis	0.003
Transplant in US vs. Foreign Center	< 0.001

9. Variables that affect the probability of Transplant-Related Events (TRE: autologous reconstitution, receiving another transplant and death from any cause). Multivariate analysis by Cox Regression.

a. All Patients

Variable	p value *
Patient's Age	< 0.001
HLA Match Level	0.003
Total Nucleated Cell Dose	0.034
Center's Experience (PCB Transplants)	0.001
US vs Foreign Center	0.082
Disease Diagnosis	0.096

b. Engrafted Patients

Variable	p value *
Patient's Age	< 0.001
HLA Match Level	0.002
Total Nucleated Cell Dose	0.7
Center's Experience (PCB Transplants)	0.2
US vs Foreign Center	0.3
Disease Diagnosis	0.096

10. Variables that affect the probability of Leukemic Relapse. (ALL, AML and CML). (Multivariate Analysis by Cox Regression).

Variable	p value *
Disease Stage	0.002
Presence of GvHD (grade III-IV)	0.053
Number of HLA Mismatches	0.16

* from Cox Regression.

C. Data.

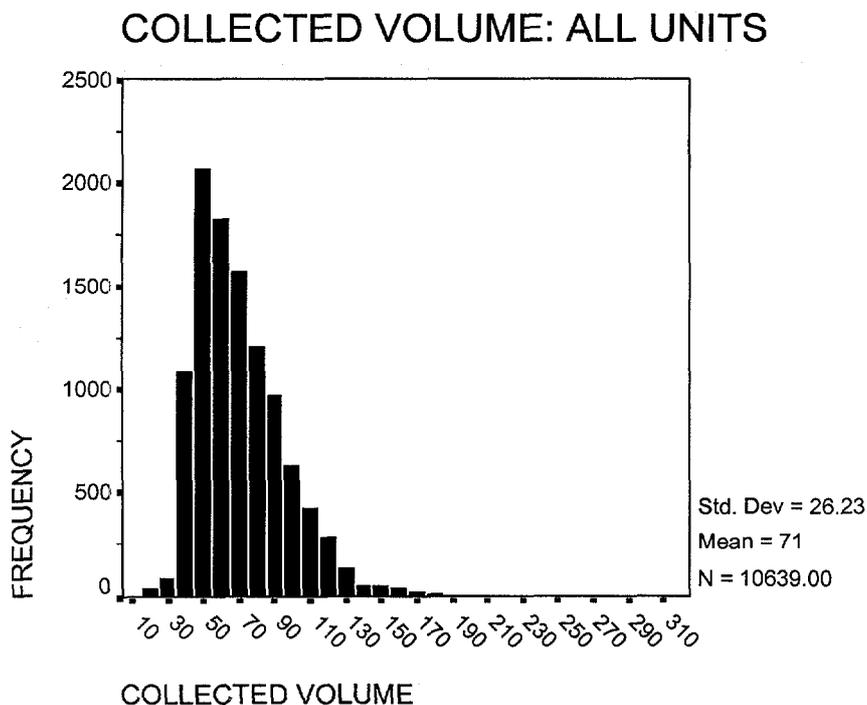
1. Collection Procedure.

From the inception of this Program, we have collected the PCB from delivered placenta. This avoids the possibility of inflicting an additional risk on the mother or child by collecting the blood during Labor. It also avoids the debate on whether collecting the blood may limit the baby's blood volume, since there is no interference with the medical management of Labor.

Initially, we studied every collection for volume and cellular content, but after the first 300 units, the lower bound of volume for retention in the Program was set at 40 ml. This volume was raised to 50 ml in November, 1999.

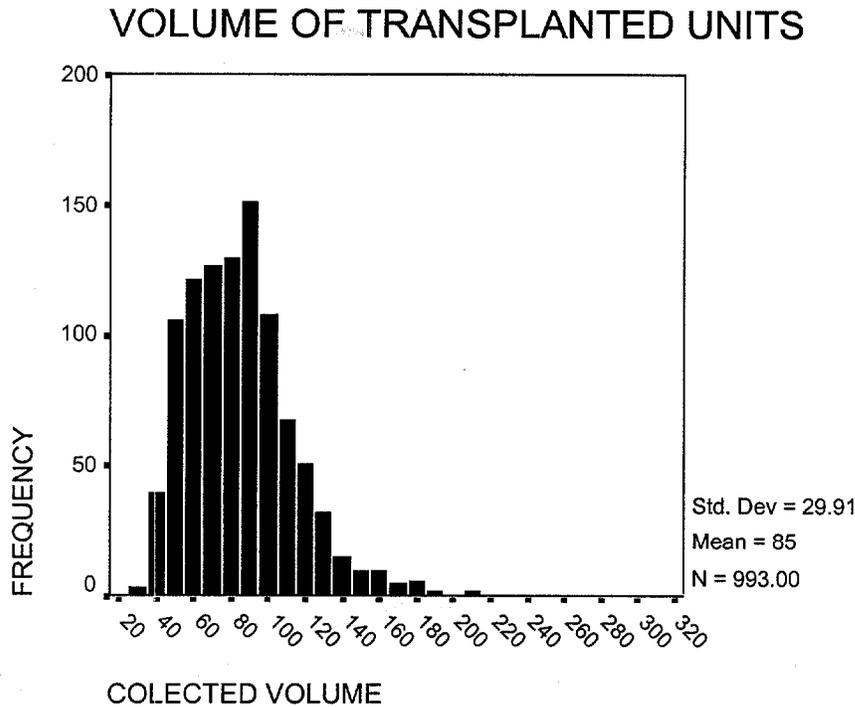
The frequency distribution of volumes collected is displayed in Fig. 1

Figure 1



We can compare this distribution with that of the units actually transplanted, shown in the following histogram (Figure 2). This comparison is the basis for future modeling and calculation of the optimal size threshold for units to be included in the inventory. Clearly, the skewness differs in the two distributions. Units of all sizes 40 ml and over were in fact used in clinical transplantation. The mode of this skewed distribution is 80 ml, reflecting the cell-dose requirements of patients of ages infant to adult.

Figure 2:



2. Processing: Methods for Volume Reduction, Cryopreservation and Freezing.

The first 3200 units were processed with the standard method for the preservation of mononuclear cells of blood and marrow: addition to the blood or marrow of an equal volume of cryopreservative solution (DMSO 20% in physiological saline). We reported in 1995 on a technique to reduce the volume of PCB units to 20ml by removing red cell mass and plasma, and then adding slowly 5ml of a concentrated cryopreservative solution containing 50%DMSO and 5% Dextran 4000. The procedure as well as the technique developed for thawing the units and removing most of the cryoprotectant solution are unchanged, despite modifications of the processing bag sets, from those in our initial report (Rubinstein, P., Dobrila, L., Rosenfield, R.E., Adamson, J.W., Migliaccio, G., Migliaccio, A-R., Taylor, P E., Stevens, C.E. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci, (USA), 92: 10119-10122, 1995.) The volume reduction technique has been used for the processing of the more recent 7300 units and has been validated at different times in the context of evaluating different plastic software for blood handling and freezing. Results of recent experiments are reported in Table 11. The viability of mononuclear cells and hematopoietic progenitors before and after freezing has been consistently excellent (>92%) as has the average recovery of CFU-C (Table 12).

Table 11. Recovery of Viable Mononuclear Cells

Exp. No.	Date	Starting WBC volume (x10 ⁶)	WBC Unit.	WBC Recovery (% start)	Exp. No.	Date	Starting WBC volume	WBC in Unit. (x10 ⁶)	WBC Recovery (% start)
1	12/14/98	79.3	713	91	20	3/7/00	127.7	2145	99
2	12/14/98	69.9	803	82	21	3/7/00	119	1404	91
3	12/14/98	94.4	698	95	22	3/7/00	94.7	1202	88
4	12/16/98	76.9	730	92	23	3/7/00	66.8	648	95
5	12/16/98	65.1	670	85	24	3/8/00	110.5	895	88
6	12/16/98	115.4	1615	95	25	3/8/00	79.6	995	104
7	12/17/98	108.4	715	80	26	3/8/00	71.3	777	100
8	12/17/98	80	752	92	27	3/8/00	74.3	520	77
9	12/18/98	84.8	881	95	28	3/8/00	91.2	1176	94
10	1/26/99	82.1	821	94	29	3/8/00	228.9	2632	91
11	1/26/99	96.6	927	91	30	3/8/00	94.9	873	86
12	1/26/99	99.7	1375	78	31	3/8/00	113.2	1189	100
13	1/26/99	84.1	672	94	32	3/8/00	97.1	1155	88
14	1/26/99	68.8	399	92	33	3/8/00	103.4	569	83
15	2/2/99	56.8	630	98	34	3/8/00	74.1	407	96
16	2/2/99	67.1	429	86	35	3/8/00	126.1	2434	98
17	2/2/99	74.4	706	98	36	3/8/00	67	482	88
18	2/2/99	73.9	872	95	37	3/14/00	68.2	464	99
19	2/2/99	174.8	1783	90	38	3/14/00	78.7	984	86
Mean:				91%	Mean:				92.10%

Table 12 describes the yields and viability of WBC and CFU-C of 13 different units after processing and after freezing and thawing using the new, closed, processing bag sets. The results have improved since then with respects to the reliability of cell recovery after volume reduction from individual samples.

Table 12. CELL VIABILITY AND % RECOVERY

AFTER PROCESSING AND FREEZING/THAWING

INITIAL DATA						AFTER VOLUME REDUCTION						AFTER FREEZING AND THAWING					
NIT	Date	Vol.	WBC No.	WBC viab.%	CFU-C	Vol.	WBC Count	WBC viab%	WBC recov. %	CFU-C	CFU-C recov. %	UNIT	Vol.	WBC Count	WBC viab. %	CFU-C	CFU-C recov.%
1	6/24/97	94	8.88	98	ND	21.7	26.62	95	70	ND	ND	1	50	8.7	56	ND	ND
2	6/27/97	100	9.55	91	1179	19.2	41.08	90	83	1160	98	2	50	9.77	29	1024	88
3	7/17/97	47.3	43.54	99	1631	20.1	94.82	97	93	1296	80	3	50	16.37	75	1192	92
4	7/18/97	61.4	8.11	98	1482	20.1	24.53	95	99	1511	101	4	50	2.45	29	broken	ND
5	7/22/97	64.7	15.38	99	3056	22	30.14	94	80	2687	88	5	50	8.6	54	2558	95
6	7/22/97	50.9	7.1	95	917	23	15.62	90	98	954	104	6	50	2.13	40	939	98
7	7/23/97	52.5	6.2	98	1214	22	28.93	89	109	1122	92	7	50	3.05	60	1148	102
8	7/24/97	51.7	10.32	99	1599	20	27.6	90	107	1485	93	8	50	3.74	36	1337	90
9	7/29/97	65.9	11.14	98	2513	22.5	29.37	89	105	3218	128	9	50	5.55	42	3153	98
10	8/1/97	72.5	9.36	91	1148	22.9	31.02	92	109	936	82	10	50	9.85	53	761	81
11	8/4/97	57.3	11.34	92	683	25.8	21.78	89	97	548	80	11	50	8.25	50	848	154
12	8/4/97	57.3	8.2	92	1219	22.1	21.78	89	102	1010	83	12	50	8	51	1031	102
13	8/5/97	62.5	11.14	93	2380	21	36.67	90	99	2345	99	13	50	9.05	39	2099	90
EAN:		64.4		96	1585	21.7	32.7	91	96.23	1523	94	MEAN:	7.347	47	1462.7	99.1	

Vol.= volume in ml, WBC counts as 10⁶/ml A1 and Hematopoietic colonies per million white cells.

Volume-reduction: effect on Engraftment. The most rigorous and useful criterion for evaluating these procedures is given by a comparison of the engraftment speed and overall probability of engraftment between the standard techniques and the modifications introduced in the 1995 article. This analysis was done using the Kaplan-Meier procedure as described in Figure 3. The significance was estimated for the difference in achieving an absolute neutrophil count of 500/ μ L of blood (ANC500). By day 42, 79% of the recipients of volume-reduced PCB grafts and 81% of the recipients of non-volume-reduced units had achieved ANC500.

Figure 3.

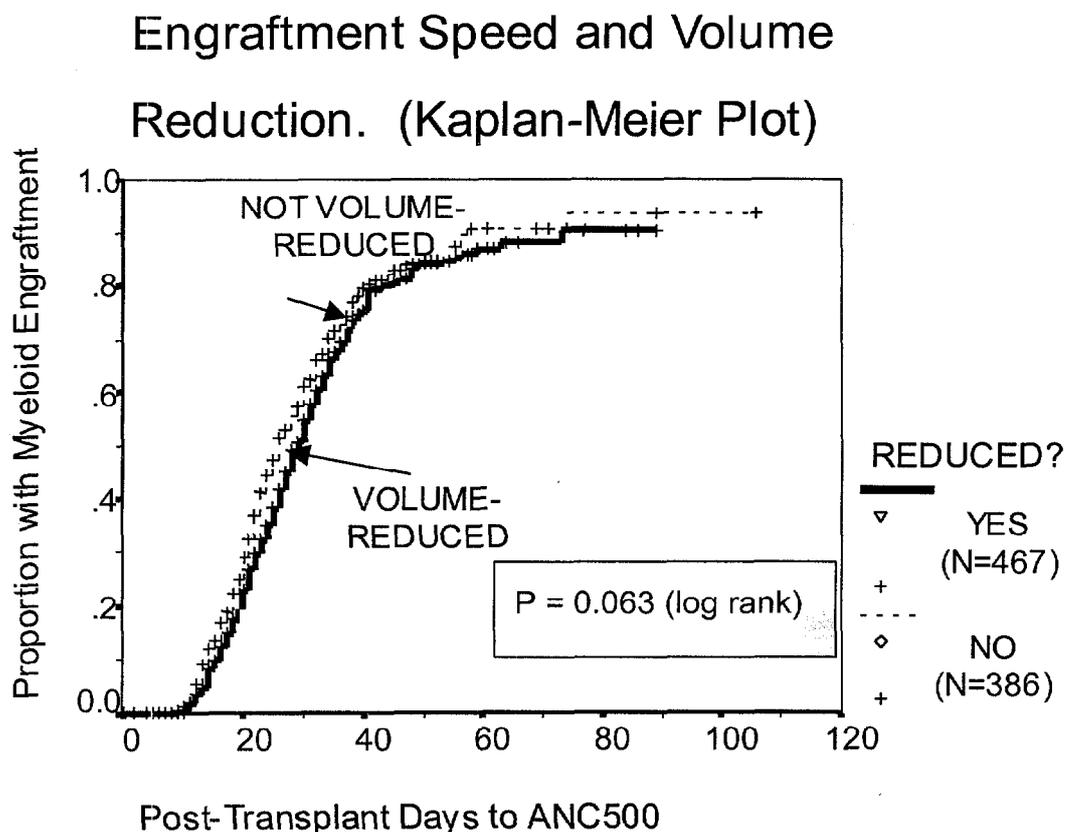


Figure 3 shows that volume reduction produces a delay of two to three days in the time to myeloid engraftment. Although the difference is small, the large number of observations included have raised its significance somewhat ($p=0.063$) suggesting that the cell losses though small are important. Further efforts to decrease the cell losses in the course of volume reduction are clearly warranted.

Volume Reduction: effects on Secondary Graft Failure? The diagnosis of secondary failure was assigned by the respective Transplant Physician to 14 patients,

seven who received volume-reduced and seven non-volume reduced grafts. There is no evidence of the possibility that the volume reduction procedure may reduce preferentially the number of "true" hematopoietic stem cells and thus compromise the long-term stability of marrow reconstitution by unrelated PCB transplants.

3. Testing and sample identification.

a) Sample Identification numbers and labels. Concern with the prevention of errors in the identification of samples, reports and test-results has been built into our procedures since the beginning of this Program. Thus, to prevent clerical errors, we developed a system based on bar-coded ID numbers that identify a mother and her child with the same number, including a suffix, M (mother) and P (placenta), respectively, as identifiers. The numbers are assigned in the Hospital as soon as the placenta is delivered to our staff. The numbered labels are acquired commercially as high-quality preprinted self-stick label sets and strict rules govern their assignment to individual samples (the main collection bag, the mother's blood sample, the data sheets, etc). One label is placed in the clinical chart, for purposes of linking the number with the mother's name. After initial testing is completed and its results reported to the mother's physician, the number will be the *sole permanent identifier*. This linkage is done by determining the birthdate of the donor and the name of the Maternity Hospital and examining the charts of all infants born in the same day for their respective ID numbers. This is a precaution designed to prevent unauthorized access to the donor's family and it has been effective in easily locating the information required in the few cases in which it was needed. Computer records are all established and updated by scanning the bar-code off specimens and forms during processing, testing and entering results. No manual entries are necessary and the reliability of the record with respect to the donor's identifier has been validated. Bar-code scanning has replaced manual entry of human readable labels throughout the Laboratory

The reliability of the label as identifier on the aliquots for testing and HLA typing has been investigated by making several different comparisons.

a) Mother-child HLA match. HLA-typing of individual alleles at each of three loci are mandatory for matching donors and recipients. Complications in the interpretation may arise because of the advances in allele definition. Class I typing (for alleles at the -A and -B loci) has been done heretofore mostly at the serological level "with splits" while DRB1*alleles have been determined almost universally by PCR-based methods at the DNA level. Continuous advances in the identification of new variants of well established alleles has rapidly changed the definition of alleles and this may cause problems when comparing the original and current typing results, if they are separated by more than a few months. This comparison, therefore, must be done judiciously. In our case, mothers and donors were tested with the same reagents initially, but the phenotype of the baby, a potential donor, may have been updated more recently. Taking this into account, we have attempted to match all mother-baby pairs at the HLA loci to identify

cases of inaccurate identifiers that are the result of errors in labeling, aliquoting or causes other than serological or technical non-specific differences in the HLA typing results.

Matching was done by flagging 6229 mother-child pairs, typed serologically for –A and –B and by PCR-SSOP and SSP for DRB1, who did not share at least one antigen at each of the three HLA loci. All blanks were considered the result of homozygosity for the paired allele. This has resulted in the identification of a total of 125 mother-child pairs with at least one apparent inconsistency (2.0%). There were 101 cases (1.6%) where the inconsistency had been due to serological cross-reactivity of one antigen, assignment of a different serologic “split” or to transcription errors, but in 24 cases (0.4%) the mother’s sample was genetically incompatible with her putative child. In 4 of the latter instances, a reciprocal exchange between the labels of two mothers who delivered on the same day that could account for the discrepancy was suggested. The search for and investigation of these 24 cases shows that despite a level of technical care consistent with current guidelines for accredited HLA typing laboratories, some, though relatively few, errors in sample identification still persist.

b) Confirmatory Class I typing from “segments”. These segments are part of the inlet, liquid nitrogen-grade tubing of the freezing bag, sealed but not cut away, in which a small volume of cryoprotected cell suspension is maintained.

The purpose of typing segments is twofold. First, to catch errors incurred when the wrong label ends up in a test aliquot (with the result that the initial typing belongs to a different unit, as in the case in our Adverse Event) and second, to correct the typing, if necessary, by using more up-to-date reagents than were available at the time of initial testing. Table 13 lists the “segments” used as a source for routine pre-transplant confirmation of HLA phenotype since 1995 (n=987) in which a change was made to the original Class I type. The “Adverse Event”, presented below, is omitted from this Table. The adjudication of discrepancies is listed under “Comments”.

Table 13. Confirmatory Typing from Segments: Class I. All typing done by the Immunogenetics Laboratory, N.Y.B.C.					
Sample	Initial Typing		Confirmatory Typing		Comments and Adjudication
	A	B	A	B	
1	2,3	58,bl	2,3	57,58	Confirmed B57
2	3,11	57,bl	3,11	41,57	Confirmed B41
3	2,24	62,bl	2,24	62,*4802	Defined Only By DNA

4	30,33	13,57	30,33	13,58	Confirmed B58
5	29,30	51,bl	29,30	78,bl	Confirmed B78
6	24,bl	40,27	24,bl	48,27	Confirmed B48
7	2,32	51,53	2,32	51,35	Confirmed B35
8	23,74	57,70	23,74	58,70	Confirmed B58
9	1,11	35,53	11,36	35,53	Confirmed A36
10	2,74	57,63	2,74	57,bl	Confirmed BL
11	1,28	13,37	1,28	47,37	Confirmed B47
12	2,28	35,53	2,33	35,53	Confirmed A28
13	2,11	60,76	2,11	60,75	Confirmed B75
14	2,19	35,44	2,74	35,44	Confirmed A74
15	2,24	51,bl	2,24	51,52	Confirmed B52
16	29,31	13,44	29,bl	13,44	Confirmed BL
17	2,3	14,57	2,3	14,58	Confirmed B58
18	2,74	57,63	2,74	57,bl	Confirmed BL
19	11,24	60,63	11,24	60,77	Confirmed B77
20	2,26	38,44	2,6601	38,44	Confirmed A6601
21	1,26	7,8	1,6601	7,8	Confirmed A6601
22	2,26	51,55	2,26	51,56	Confirmed B56
23	1,11	35,53	36,11	35,53	Confirmed A36
24	29,31	13,44	29,bl	13,44	Confirmed BL
25	2,bl	44,50v	2,bl	44,4005	Confirmed 4005
26	1,bl	8,49	1,24null	8,49	Defined Only By DNA
27	2,26	51,52	2,6601	51,52	Confirmed A6601
28	1,2	8,63	1,2	8,*1524/36	Defined Only By DNA
29	3,23	49,70	3,23	49,50	Confirmed B50
30	2,bl	57,70	2,bl	57,35(short)	Confirmed B35(short)
31	2,33	70,bl	2,74	70,bl	Confirmed A74
32	1,26	8,58	1,6601	8,58	Confirmed A6601
33	1,34	8,53	1,6602	8,53	Confirmed A6602
34	2,30	7,bl	2,30	7,42	Confirmed B42
35	29,30	51,44	29,30	78,44	Confirmed B78
36	25,34	18,38	25,66	18,38	Confirmed A66
37	2,34	27,41	2,6601	27,41	Confirmed A6601

38	1,31	8,bl	1,31	8,39	Confirmed B39
39	24,28	39,53	24,28	39,bl	Confirmed BL
40	2,28	35,61	2,28	35,48	Confirmed B48
41	1,30	53,42	36,30	53,42	Confirmed A36
42	1,33	8,60	1,*2607	8,60	Defined Only By DNA
43	2,bl	44,bl	2,bl	44,*5104	Defined Only By DNA
44	11,26	35,60	11,6601	35,60	Confirmed A6601

There were 44 phenotype changes suggested but one was not accepted on retyping. All the 43 changes (4.4%) were either for phenotype refinement or non-expressed antigens rather than correction of errors. A single error of sample identification has been encountered, which resulted in the Adverse Event (copied below from the original report to FDA).

c) Inter-Laboratory confirmation of HLA-typing results. During the same period we have submitted material from 1781 samples for confirmatory typing by Transplant Center Laboratories and have received 1616 samples from transplant candidates for confirmatory typing here. There were 27 discrepancies of the first type (1.52%) and 19 of the second (1.17), all were adjudicated in collaboration with the outside Labs. The small number and the general character of the changes indicate that typing is generally accurate but that errors still occur and confirmation is still required.

Adverse Event Report.

BB IND 6637

Name of Drug: Placental/Umbilical cord Blood

Indication Covered: Bone Marrow Reconstitution

Date of original submission: April 26, 1996

Principal Investigator: Pablo Rubinstein, M.D.

Description of the Event.

A 2-year old Italian patient suffering from relapsed infant acute lymphoblastic leukemia in 2nd complete remission, ostensibly unable to obtain a conventional bone marrow donor, requested a placental/umbilical cord blood unit for transplant from this Program on 9/17/98. A unit matching four of the patient's HLA six antigens was proposed and accepted for the Transplant. The unit's typing was confirmed locally by a study of the serological HLA antigens and a DNA sample from it was sent to the Transplant Center for HLA type confirmation there. The patient's HLA type was confirmed in our Laboratory from a patient's blood sample and the proposed unit's type was confirmed in Italy from the DNA submitted.

The patient was transplanted on December 10th, 1988, engrafted on 1/11/99 and by 1/18/99 had an ANC=3.9x10⁹/L, with mixed chimerism. Despite GvHD prophylaxis and therapy, the patient developed grade 3 GvHD of the skin. A possible lung alteration is mentioned. We received this information on May 18 with the request that we investigate the possibility of a mistake, because of discrepancies in the typing. The patient's typing results as well as those of other units collected on the same day (and thus, aliquoted for testing, cryoprotected and frozen on the same date) were reviewed in New York and compared with those obtained earlier and with the post-engraftment types of the patient and an error was detected. The aliquot samples of the unit in question and those of another of the same date's units were switched and thus the HLA typings done on these aliquots did not correspond to the units whose bar-coded numbers they carried. While our testing was in progress, the patient was again ablated and the back-up (autologous marrow obtained when the patient was in complete remission) reinfused on 5/18/99. The patient subsequently engrafted and is currently in complete remission with apparently complete repopulation from the recipient.

Investigation of the Event.

The report of a possible error was received in 5/14/99, with a request from Dr. Locatelli for help in determining the cause so that improved safeguards could be put in place to prevent future situations of this type.

The patient's HLA type was: A26,32; B18,38; DRB1*1104,1301 and the unit transplanted (No. 100464) had HLA type: A2,26; B18,38; DRB1*01104,1301. The match grade was thus 4/6.

The typing after engraftment (1/18/99) was reported from Genoa only as A2,26,32 and a retrospective complete typing of the same sample, performed on 5/8/99 confirmed A2,26,32 and added B7,18,38 and DRB1*4,11 and 13, indicating mixed chimerism with the unexpected addition of B7. A new typing performed on 4/28/99 showed HLA-A2,26; B7,38; DRB1*0402,0405, which differed from the expected donor typing in one HLA B antigen (B7 instead of the expected B18) and in both DR alleles. This last typing, also reported from Italy, was taken as confirmed although we could not ourselves type that sample.

Given that the patient was reportedly transfused only with filtered, leukocyte-poor blood and blood components, we investigated the possibility of a mistake in our operations. Processing is done with only one unit in the laminar-flow hood at a time, under a specific SOP, to guarantee the integrity of the labeling of aliquots from that unit. There are other possible steps in which a random mistake might occur, but unit No. 100465, collected in the same day as 100464, had the exact HLA phenotype found in the patient's typing of 4/28/99, after the complete engraftment of the transplant. In fact, the HLA type of unit 100465 was: A2,26; B7,38; DRB1*0402,0405. In our protocol, before a unit is released to the Transplant Center, the class I antigens are again tested in the

cells contained in the segment, a length of the inlet tubing containing a small volume of cryoprotected placental blood, sealed but still attached to the bag. This typing should have demonstrated the presence of different antigens, enabling us to discern the error. However, three of the four A,B antigens in this case were identical and the fourth, B7 rather than B18, resulted in a cross reaction in which all anti-B18 sera were reactive while only some of the B7 showed this cross reaction. The rest of the typing antisera behaved according to expectations and the graft was therefore authorized and dispatched.

Interpretation of the results.

1. *The event was due to an error that occurred in the third month of operations in this project, in which two units must have been processed together, in direct violation of the SOP. As a result, the aliquots of 100464 were labeled as 100465 and vice-versa. Thus, the HLA phenotypes of the grafts were reversed.*
2. *An additional error was due to the very close HLA class I antigenic similarity of the two donors of these units, born on the same day. There was also an error in not investigating the apparent cross-reaction between B7 and B18, antigens not classically recognized as cross-reacting. This prevented us from recognizing that the unit under consideration did not fit accurately the reaction patterns expected.*

To guard against repetition of this event, we then changed our procedures.

- a) segment typings are now repeated by PCR-SSP typing in all cases when serology does not provide a clear and unambiguous phenotype.
- b) The HLA phenotypes of all babies whose PCB was processed at the same date are evaluated prior to releasing the sample, and
- c) the use of multiple gene polymorphisms as a genetic signature for the donor in the initial sample and in the segment material is being validated. Although it is a rather expensive system, the capacity to detect sample mistakes is much larger than using HLA, particularly if only Class I typing is used.

4. Analysis of the hematopoietic potential of PCB grafts.

The engrafting capacity of hematopoietic transplants is now known to depend on their stem cell content. Since stem cells are not yet quantifiable directly in clinical materials, however, surrogate methods are used universally for this purpose. In the case of PCB, the most widely used criterion is the number of nucleated cells or the mononuclear cells of the grafts. There is, however, substantial experimental work that suggests a better surrogate may be found in the colony-forming cells (CFU-C). CFU-C tests are laborious and require prolonged (2 weeks) incubation, and our associates in this work were unable to culture all units.

For that reason, we determined the number of CFU-C in all PCB units with volume under 60 ml and about 1/3 of the total collections. More recently, in collaboration with

Dr. Jan Visser, we have explored the possibility of using the CD34 marker in PCB collections. Finally, as detailed elsewhere in this report, we have found that the numbers of immature erythroid cells (nucleated red blood cells or erythroblasts) are also related to the speed of engraftment.

a) Total nucleated cell (TNC) and colony-forming cell (CFC) dose. The total number of nucleated cells in the graft is classically related to the overall success of bone marrow transplantation. In fact the relative paucity of these cells in PCB grafts has been a factor against their use in many adult recipients. The TNC dose per Kg of recipient body weight is generally used as criterion and in the case of PCB, it shows good correlation with engraftment speed for both neutrophils and platelets. We have analyzed the correlations between these two indices and the speed of engraftment of myeloid cells and platelets in the Attached manuscript. (Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM and Rubinstein P. "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, 2000, in press). Interestingly, the cell doses before freezing were more closely correlated with the speed of engraftment than those after thawing (data from Transplant Centers). The uni- and multivariate correlations are shown in Table 14.

Table 14.

Relative Risk of Engraftment Endpoints and Transplant-related Events (TRE).

Variable	Number of Patients	Relative Risk (95% CI)	
		Univariate (95% CI)	Multivariate (95% CI)
<u>Myeloid Engraftment (Time to Absolute Neutrophil Count \geq 500/μL):</u>			
TNC per Kg (pre-cryopreservation, $\times 10^6$):			
≥ 100	21	4.8 (2.8 – 8.4) P<0.001	1.5 (0.6 – 3.9) P=0.4
50-99	41	1.7 (1.1 – 2.8) P=0.024	0.7 (0.4 – 1.3) P=0.3
25-49	67	1.8 (1.2 – 2.8) P=0.006	1.2 (0.8 – 1.9) P=0.4
< 25	61	1.0	1.0
CFC per Kg (pre-cryopreservation, $\times 10^3$):			
≥ 200	28	5.2 (3.0 – 9.0) P<0.001	4.7 (2.0 – 11.1) P<0.001
100-199	43	4.2 (2.5 – 7.0) P<0.001	4.7 (2.6 – 8.8) P<0.001
50-99	61	2.4 (1.5 – 3.8) P<0.001	2.4 (1.5 – 3.9) P<0.001
< 50	58	1.0	1.0

Table 14. (Continued)

Platelet Engraftment (Time to Platelet Count \geq 50,000/ μ L):

TNC per Kg (pre-cryopreservation, $\times 10^6$):			
≥ 100	21	2.0 (0.99 – 4.0) P=0.052	0.4 ((0.1 – 1.3) P=0.12
50-99	40	0.9 (1.3 – 4.6) P=0.005	1.1 (0.5 - 2.4) P=0.9
25-49	64	1.1 (0.6 – 2.0) P=0.8	0.8 (0.4 – 1.5) P=0.5
< 25	55	1.0	1.0
CFC per Kg (pre-cryopreservation, $\times 10^3$):			
≥ 200	28	4.4 (2.1 – 9.1) P<0.001	8.1 (2.7 – 24.2) P<0.001
100-199	40	3.5 (1.8 – 6.9) P<0.001	4.0 (1.8 – 8.7) P=0.001
50-99	59	2.0 (1.1 – 3.9) P=0.034	2.0 (1.0 - 3.9) P=0.037
< 50	53	1.0	1.0

These data indicate that the Relative Risks associated with engraftment speed, which are dependent on the cell dose, correlate better with CFC than TNC. In fact, the introduction of CFC into the multivariate model dissipates the significance of the TNC.

b) CD34 as a marker of hematopoietic progenitor-cell activity. In the last year, a collaborative effort with Jan Visser has resulted in an improved definition of the hematopoietic subset of CD34+ cells in PCB. Dr. Visser submitted the data to the 1999 ASH meeting (JWM Visser, GE Alespeiti, CE Stevens and Pablo Rubinstein RELATION BETWEEN NUMBERS OF CD34+ CELLS AND CFU-C IN PLACENTAL CORD BLOOD UNITS. Blood (1999) 94, no 10, Supplement 1 part 2, page 350b, Abstract # 4792). This report disclosed the existence of a staining difference between the hematopoietic progenitor cells and a more intensely staining CD34+ endothelial-like cell population without hematopoietic function. The less intensely staining cells had a very strong numerical correlation with the CFC determination on the same PCB unit. These data may help the development of a more efficient method for estimating the hematopoietic potential of individual PCB units. The complete Abstract is reproduced in the next page).

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RELATION BETWEEN NUMBERS OF CD34+ CELLS AND CFU-C IN PLACENTAL CORD BLOOD UNITS. Jan W.M. Visser, Gabriel E. Alespeiti*, Cladd E. Stevens* and Pablo Rubinstein*, Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY.

The engraftment potential of placental cord blood (PCB) units can be largely predicted from their CFU-C content (Rubinstein et al, manuscript in preparation). Although CFU-C are not identical to pluripotent hemopoietic stem cells (PHSC), their incidence in each unit must be in close balance with that of the PHSC. An alternative method to count a cell type closely related to PHSC could be flow cytometry using the CD34 marker. Published data on CD34 counts in PCB units are not in agreement, probably because of technical difficulties. Standard kits are commercially available to count CD34+ cells. We tested one of these (ProCOUNT, Becton Dickinson) on 48 freshly obtained PCB units and compared the CD34+ counts with the CFU-C numbers for 43 of these.

Absolute counts of CD34+ cells in these units ranged between 9.7 and 110 cells per μ L, the average being 37.4 cells per μ L. The CFU-C counts ranged *between* 4.3 and 95.8 per μ L with an average of 19.4 per μ L. Application of the standard ProCOUNT protocol resulted in a correlation coefficient of 0.87 between CD34+ and CFU-C numbers. However, the standard CD34 labeling resulted in two clusters of CD34+ cells, a CD34-intermediate and a CD34-bright population. The ratio of the cell numbers in these two clusters was different per sample. On average 17 % of all CD34+ cells belonged to the CD34-bright population, but the range varied between 2.2 and 53 %, In view of this we also examined the relation between the numbers of these cell types in each of these clusters separately with those of CFU-C. The correlation coefficient between CFU-C and the CD34-intermediately positive cells was found to be 0.91, whereas that with the CD34-bright cells was -0.024 .

The nature of the two CD34+ cell types is currently being investigated using additional markers. Our results indicate that only the number of CD34-intermediate cells is closely related to that of PHSC and we speculate that the CD34-bright cells are circulating endothelial cells or their precursors. The presence of such cells in cord blood has been described before. Our present data indicate that the incidence of the latter is not correlated with that of PHSC, and therefore, that the mechanisms by which the various cell types are released into the circulation are probably different.

5. Bacterial Contamination.

Not surprisingly, it is easy to contaminate the PCB with bacteria during collection. We now present our strategy, methods and experience in evaluating this problem. It should be remembered, however, that the problem is not unique to PCB and that it has been frequently occurred with surgically collected bone marrow.

- a) **Strategy for bacterial culture.** PCB units are cultured under aerobic and anaerobic conditions using an automated system (ESP Blood Culture System). Aliquots from each PCB unit (0.5 ml for aerobic culture and 0.1 for anaerobic), inoculated into culture bottles containing aerobic and anaerobic media, are incubated on the instrument for 7 days followed by incubation off the instrument in a 37° C incubator for another 7 days. Cultures that are reactive on the instrument or are turbid at the end of 14 days are sent for subculture to identify the organism and establish antibiotic sensitivity. In addition to routine culture, we also cultured a subset of PCB units at various time points during processing to assess the reproducibility of the culture results and the possibility of contamination during processing.
- b) **Routine bacterial culture results.** Among 10,517 PCB units with bacterial culture results completed to date, 240 (2.2%) had either a positive reaction on the DIFCO System (n = 232) or turbidity after 14 days incubation (n = 8). A full analysis of the data is presented in the attached appendix (A). In summary, the data demonstrated the following:
1. Altogether, 19% of reactive cultures failed to demonstrate bacterial growth on subculture, somewhat more frequently in reactive anaerobic cultures.
 2. About one-third of positive cultures were detected only by aerobic culture, one-third only by anaerobic culture and the remainder by both methods.
 3. The prevalence of confirmed culture positivity (organism detected on subculture) was strongly influenced by the technique used to clean the umbilical cord prior to collection and inversely by the volume collected. Smaller volume units were generally more difficult to collect, taking longer and, at time, requiring multiple sticks.
 4. The prevalence of confirmed culture positivity was associated with route of the infant's delivery. There was also a trend toward an association with a history of chorioamnionitis and maternal fever during labor, although these associations did not reach significance.

c) **Culture at various times during processing.** In February 1995, we introduced a method to remove excess red blood cells and plasma from the unit thereby reducing the final frozen blood volume and the amount of DMSO required. In a series of studies, we explored the possibility of bacterial contamination during processing as well as the most appropriate time to obtain an aliquot for bacterial culture. In these studies, aliquots for culture were taken from the whole PCB unit prior to processing as well as at various post-processing stages. In the first study (6/27/97 to 1/27/98), 643 PCB units were cultured pre-processing and just prior to the addition of DMSO. Altogether, cultures were positive on 17 units, 2 only on the pre-processing aliquot, 10 only on the post-processing aliquot and 5 on both. Among the 10 samples positive only post-processing, 5 were positive for *Propionibacterium acnes*, an organism that was not often found in PCB units but also cultured from one technician's hands (this technician was excluded from participating in the subsequent studies). In the second study (1882 units, 1/28/98 to 5/31/99), aliquots were taken for culture pre-processing and just after adding DMSO. Cultures were positive on a total of 36 units, approximately equal numbers only pre-processing, only post-processing and both pre- and post-processing. In the final investigation (356 units, 6/1/99 to 9/5/99), the post-processing specimen was taken from the plasma removed after addition of DMSO in the final step of volume reduction prior to freezing. Because there was relatively no limit to the volume of plasma available, we increased the volume cultured to 1.0 ml for both aerobic and anaerobic culture. In this study, the culture was positive on the pre-processing aliquot in 3 cases, in the plasma only in 1 case and both pre and post-processing in 3 cases. Altogether, these studies suggested the following conclusions:

1. there was no suppressive effect of DMSO on the system's ability to detect bacterial contamination.
2. Many of the culture positives were not detected in both pre- and post-processing aliquots. These discrepancies could be due to missed detection because of the limited volumes cultured (sensitivity affected by volume cultured) or to contamination of the unit during processing (for those that were positive only post-processing) or to contamination of the culture bottle during inoculation (a possible explanation for any of the discordant cultures).
3. There was no difference, overall, in the positivity rates pre- and post-processing, suggesting that little, if any contamination of the units occurs during processing.
4. There was no apparent improvement in detection of bacterial organisms by culturing at different stages of processing.

In view of these results, we elected to stop culturing the whole PCB unit prior to processing and to culture only an aliquot taken from the plasma removed in the

final processing step. This strategy is logistically simpler than taking an aliquot at any other step, has the advantage of not delaying freezing and gives comparable results to cultures of pre-processing aliquots.

6. Nucleated Red Blood Cells (nRBC).

- a) **Goals of Evaluation.** Beginning in 1999, we undertook an evaluation of the significance of nucleated RBCs in PCB units. Recognizing that nRBCs are frequently present in cord blood and to varying amounts, several investigators have become concerned about the implications of large numbers of these cells. The concerns related to potential inaccuracy in enumeration of total nucleated cells (TNC) (most automated instruments can not identify nRBCs and count them in the mononuclear cell subset), their impact on engraftment potential and their lysis on thawing with consequent high loss of TNC. To assess these issues we retrospectively examined peripheral PCB smears taken on units at the time of collection to enumerate nRBCs. PCB units selected were all units transplanted through the end of December 1999 and, to obtain a more representative sample, a random subset of non-transplanted units, stratified by ethnicity. Counts, made of 100 nucleated cells distinguishing between white blood cells (WBC) and nRBC, were used to calculate the number of WBC and nRBC in the unit and, for transplanted units, the dose of WBCs and nRBCs per kilogram recipient body weight. Full data from this study is shown in Appendix B. A summary and conclusions are presented below.
- b) **Data.** The number of nRBCs in PCB units varied widely, following a Poisson distribution with a mean of 8.4 per 100 WBCs or 1,013 per microliter. nRBC counts correlated with TNC, colony forming units (CFU) and with CD34+ cell counts. As with TNC or WBC dose, nRBC dose correlated with speed of myeloid engraftment (time to ANC 500) and event-free survival. In a multivariate analysis, nRBC and WBC dose were independent predictors of engraftment speed. When CFU dose was included in the analysis, however, only CFU dose predicted the speed of engraftment and neither WBC nor nRBC dose was significant.

To assess the significance of nRBCs in PCB units, we also examined possible risk factors for elevated nRBC counts (i.e., > 2 Standard deviations above the mean or $\geq 3,000/\mu\text{L}$). As previously reported, high nRBC counts were associated with potential causes of hemolysis in utero (ABO incompatibility), fetal hypoxia (fetal distress in utero, meconium staining, maternal smoking during pregnancy), maternal fever during labor, birth weight and maternal diabetes mellitus. Interestingly, a high proportion of infants born to African American women had high nRBC counts, even after adjusting for other recognized risk factors, an observation that requires further exploration.

Because nRBC number correlates with CFU, we also examined the relationship between the above risk factors and CFU counts. CFU count also correlated positively with ABO incompatibility, birth weight and maternal fever, but was significantly lower in PCB units from African Americans (after accounting for the other correlates) and when the mother was a cigarette smoker, data suggesting the possibility of shared as well as distinct mechanisms affecting nRBC and CFU production and/or release into the circulation.

Based on the above data, we undertook an evaluation of the Sysmex XE-2100, an automated hematology analyzer (Roche Diagnostics, Inc.) advertised to count nRBC reliably. In this evaluation, nRBCs were counted from peripheral blood smears from 248 sequential PCB units under code by three technicians and counted, in duplicate, by the XE-2100. Manual nRBC counts correlated strongly between individual technicians (R squared = 0.72-0.78, $P < 0.001$) as did the instrument duplicates (R squared = 0.99, $P < 0.001$). Moreover, the correlation between the average manual counts and the instrument counts was also excellent (R squared = 0.80, $P < 0.001$).

- c) **Conclusion.** We conclude from these data that high levels of nRBC should not preclude use of PCB units for transplantation. In fact, units with high nRBC counts had improved engraftment potential, because of their association with number of hematopoietic progenitors, compared with units containing the same TNC count and few nRBCs. While these data suggest that TNC count need not be corrected for nRBC, enumeration of nRBCs is potentially useful to the transplant physician because of their preferential loss on thawing. Given the excellent correlation between manual and automated nRBC counts, either method could be used, after validation.

7. Donor Eligibility and Infectious Disease Exclusion Criteria.

The keystone of PCB banking is the development of adequate criteria to determine the usefulness of a particular PCB unit.

- a) **Strategy.** In the NYBC Placental Blood Program, we have had only a limited number of *a priori* exclusion criteria (i.e., known HIV or HBC infection, still birth, lack of maternal consent, known presence of a serious transplantable genetic disease and a minimum collected blood volume). Instead, we collected, and retained for inventory, PCB units regardless of the presence of other potential risk factors with the understanding that information on the potential risk of a transmissible disease would be provided to the transplant physician and used in selecting the most appropriate PCB units and in weighing the potential risks and

benefits of the patient. We view this strategy to be an interim approach until the inventories of PCB units world wide is adequate to supply all patients with a suitable unit having no history of risk factors. We also believe that this strategy remains especially appropriate for ethnic groups that have a high level of HLA diversity and, consequently, have difficulty finding suitable related or unrelated bone marrow or peripheral blood donors. Additionally, assessing the potential risk for a blood transmissible infection must take into account both the mother's risk (she is the conduit for transmission of infection to the infant) as well as the frequency of transplacental passage, *in utero* or peripartum. Thus, in some infections, while the mother may be acutely or latently infected, *in utero* transmission may be uncommon (as with Cytomegalovirus) or even extraordinarily rare (as with Epstein-Barr virus).

Because of this approach, allowing women with potential risks to donate their infant's cord blood, we collected detailed data on potential risk factors for blood transmissible infection (paralleling those asked of routine blood and tissue donors) as well as for family history and risks of genetic disease, especially those affecting the hematopoietic system. The current data form provides for recording data about potential risks as well as additional information to help assess the potential risk (see attached). Data is also available on serologic testing for hepatitis B virus markers (HBsAg and anti-HBs), hepatitis C virus (anti-HCV), human immunodeficiency viruses type 1 and 2 (anti-HIV-1, 2 and HIVp24Ag), human T-lymphotropic viruses type I and II (anti-HTLV-I, II). Generally, both mother's and PCB plasma samples were tested for these viral markers. In addition, we tested mothers and a subset of PCB units for Cytomegalovirus (CMV)-specific IgG and IgM antibody and, beginning in 1995, cultured saliva from all infants for CMV (courtesy of Robert Pass, University of Alabama, Birmingham). Studies to detect CMV by gene amplification (PCR) in plasma and cellular DNA are currently underway. A detailed analysis of the data obtained thus far is available in Appendix C. A brief summary follows.

b) Data. Altogether, 21.7% of the women who donated the placental/cord blood of their newborns to our Program reported having a blood or STD (sexually transmitted disease) risk factor that might have excluded them from donating blood for transfusion. The prevalence of such a risk was significantly higher in Asian and African American women than in either Hispanic or Caucasian women. Among those who reported any blood or STD risk, the specific type of risk varied considerably by ethnic group. Among African American and Hispanic women, their risk was more likely than the others to be exposure to blood transfusion, tattooing or body piercing or a history of a recent STD. Among Caucasians, the most frequently reported risk was a history of hepatitis or sex with an injection drug user. Among Asian women, the most common risk was blood contact, usually through work as a health care professional. While data on such potential risks was reported to the transplant physicians, the presence of a risk factor had

little impact on unit selection. The presence of a reported risk factor had no impact on transplant outcome (engraftment or event-free survival).

As expected, the prevalence of HBV markers (either HBsAg or anti-HBc) was highest in women of Asian ancestry and lowest in Caucasians, but also varied considerably by the mother's birthplace probably reflecting the high incidence of childhood infection in some regions of the world. Anti-HCV prevalence was low (0.5%), overall, and although lowest in Asian mothers, ethnic differences in prevalence were not significant. Anti-HIV prevalence was also low (0.2%), probably reflecting, in part, staff efforts to avoid collecting PCB units from known HIV-infected mothers. Anti-HTLV prevalence was only 0.2%, overall, but has been detected, thus far, only in African American or Hispanic mothers. A relatively high proportion of mothers' plasma were repeat-reactive by the anti-HTLV EIA and indeterminate by Western blot (0.6%). A small number of these HTLV indeterminate samples have been tested by PCR and all were negative.

The prevalence of CMV-specific IgG antibody positivity was 61.7% and varied significantly by ethnic group (from nearly 90% in Asian mothers to less than 50% in Caucasians). Several assays have been used to detect CMV-specific IgM antibody in our study. When mother's plasma was tested by more than one method, a high frequency of discordant results were obtained. Of 1553 maternal specimens tested by both Abbott's previously available EIA and on Abbott's currently available IMX System, IgM positivity was detected by both methods in 2.5%, but only by one or the other assay in 6.7%. Among 6075 saliva cultures for CMV, only 12 (0.2%) were positive. CMV-specific IgM antibody (positive or borderline) was detected in about half of the mother's plasma from these cases.

HBV, HCV, HIV and HTLV confirmed seropositivity correlated with the mother's reported risk for blood transmissible infection or STDs. The prevalence of repeat-reactive results that were not confirmed as positive by RIBA-3 or Western blot, however, did not correlate with reported risk factors.

Among the PCB units provided for transplantation, none was positive for antibody to HIV or HCV. Transplant physicians chose to transplant three units whose mothers had an indeterminate HTLV western blot but were negative by PCR. Follow up is not yet adequate to assess transmission. Forty-two patients received PCB units that were positive for anti-HBc, 90% of which were also anti-HBs positive. There has been no evidence of HBV transmission from these units. CMV-specific IgG antibody was detected in nearly 60% of transplanted PCB units. CMV-IgG positivity in the PCB unit had no impact on the transplant outcome, including event-free survival and the incidence of acute CMV infection post-transplant (correlated with the patient's CMV antibody status, but not with the PCB unit's). Five units from mothers with CMV-IgM antibody were

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transplanted. One of these patients had a CMV infection post-transplant, but this patient was also anti-CMV positive before the transplant.