Cellular, Tissue and Gene Therapies
Advisory Committee Meeting
September 22, 2011

BRIEFING DOCUMENT

BLA 125397
Hematopoietic Progenitor Cells, Cord (HPC-C)

NATIONAL CORD BLOOD PROGRAM

New York Blood Center
National Cord Blood Program
45-01 Vernon Blvd
Long Island City, NY 11101

Briefing Document for the
Cellular, Tissue and Gene Therapies Advisory Committee

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1.0 BRIEF HISTORICAL REVIEW – MANUFACTURING METHODS

The National Cord Blood Program (NCBP, initially called the Placental Blood Program) was the first public cord blood bank to collect, process and store CB grafts for transplantation to unrelated recipients. Officially proposed in 1989 [1] the Program started cord blood collections for clinical use at Mt. Sinai Hospital, in February 1993, after several years of preparation and development of methods for cord blood collection and cryopreservation, and within six months of receiving a 3-year Demonstration Grant from NHLBI, NIH, September 30th, 1992. The method at that time was that the whole cord blood unit (CBU) was frozen after cryopreservation with DMSO to 10% and maintained under liquid nitrogen (LN) in conventional Dewars [2]. We are referring to this method as "Manufacturing Method 1".

The first two clinical transplants with (our) unrelated cord blood into myeloablated recipients were performed by Dr. Joanne Kurtzberg, at the Pediatric Bone Marrow Transplantation Service, Duke University Medical Center, in August and September of 1993. [3,4].The two recipients, young children with acute leukemia, engrafted and one of them is alive and well, with full-donor chimerism, in 2010. The NIH Grant supported over 5,000 cord blood collections and more than 100 transplantations through the end of 1995. During that period, the Program demonstrated that banked cord blood achieves tri-lineal marrow reconstitution when transplanted to myeloablated, HLA-mismatched, unrelated recipients. [4,5,6]

In August 1994, we changed our CBU manufacturing technique: we initiated a procedure to reduce the volume of cord blood units prior to cryopreservation. This permitted us to standardize the volume of cryoprotected cord blood units to 25 mL, prior to freezing thus enhancing our capacity to store large number of CBUs in standard liquid nitrogen Dewars. This procedure was published in the Journal of the National Academy of Sciences [7] and is referred here as "Manufacturing Method 2".

In May of 1996, when we had provided CBUs for 106 recipients from among 1432 patients for whom search requests had been submitted, we applied for and were granted an FDA IND exemption (BB IND 6637), which allowed us to continue to explore this experimental treatment and recover the costs. Quickly thereafter, major parts of the annual costs of the Program were supported by reimbursement fees, which enabled us to expand the Inventory of frozen CB units,

that were HLA-typed, screened and tested for markers of the major blood-transmissible infectious diseases, and available "upon demand". Also in 1996, the NCBP collection site was transferred to Brooklyn Hospital, attempting to correct or ameliorate the ethnicity differences in access to transplantation: Brooklyn Hospital census was >80% African American and Hispanic, while at Mt. Sinai Hospital Caucasians were ~60% of the total.

From 1994 on and responding to requests, NCBP invited US and European investigators to visit the Program and learn the procedures it used and soon afterwards additional cord blood banks were organized elsewhere in the US (Glennon Hospital, St Louis, MO and University of Colorado, Denver), Europe (Dusseldorf, Barcelona, Milano, Leiden) and Japan. The NCBP was one of the founding members (1997) of the NETCORD Organization (the other members were the cord blood banks of Milano, Düsseldorf, Barcelona, London, Leiden and Tokyo). Efforts to draft a set of Cord Blood Standards for the Accreditation of Cord Blood Banks started at almost the same time, and a few years later, the NCBP Standards were further augmented by other NETCORD Banks and provided to FAHCT (now FACT) resulting in the first Accreditation Standards administered by a recognized Accrediting organization (2001). NCBP was the first cord blood bank to be inspected by and receive FACT Accreditation (2002-2003). New NCBP collection sites were developed at North Shore Hospital in Manhasset, NY (1999), Inova Fairfax, VA and New York Presbyterian Hospital (2001).

Retrieval of transplant outcome data proceeded with the interest and cooperation of most participating transplant centers in the US and abroad and in 1998, in a report in the New England Journal of Medicine [8] we summarized information from 562 pediatric and adult patients (consecutive, representing over 90% of all NCBP cord blood grafts at the time). This information was included in our report to the FDA Workshop on Cord Blood in 1998. The raw data from these transplantations was later updated and then submitted to the FDA docket No. 1997N-0497. The information in the docket supports the clinical utility of frozen, partially-HLA-matched, unrelated, minimally-manipulated cord blood grafts, for hematopoietic reconstitution in patients with hematological malignancies, immunodeficiencies, inherited genetic and metabolic diseases, bone marrow aplasia and other diseases.

It is important to note that the information in the published report and in the FDA Docket, derive from transplants performed with CBUs from our Method 1 and Method 2 cohorts. Since the corresponding outcome data was thought to display efficacy acceptable for licensure, it can be postulated that CBUs processed by methods 1 and 2, were safe and effective, although the methods used originally to test them would not satisfy all the requirements of current GMPs.

In 1999, ThermoGenesis introduced the BioArchive, a revolutionary robotic freezer that included controlled-rate freezer modules and permitted freezing cryoprotected 25 mL cord blood units within its storage hold, and a new, two-compartment freezing bag. The BioArchive freezer and the disposable freezing bags permitted control of the previously unavoidable Transient Warming Events (TWE) encountered while transferring CBUs, frozen in controlled-rate-freezing chambers, to their storage slots in the racks of conventional Liquid Nitrogen freezers. Because of the robotic arm's capability to pull out a single unit instead of a whole rack including up to six slots, the CBUs stored in a BioArchive avoid the large number of TWEs endured by the average unit stored in a conventional freezer, which can number up to 7, or more (if the slots are re-used after withdrawing one or more units). There is some evidence that retrieving a unit from its place under LN and exposing it to temperatures of -100°C or higher, for as little as one minute, can

produce small but cumulative injury to the CBUs. That damage is completely avoided by the BioArchive, the main feature of our 3rd method for manufacturing, which further introduced the use of a "quarantine overwrap bag". The overwrap tightly binds together the bag's two compartments with the in-line segments and reduces the difference of the rates of heat escape (cold invasion), which, for unprotected segments, makes the rate too fast and decreases the viability of cells. The rationale for freezing CBUs in a two compartment bag was to allow the removal of the small compartment without thawing the larger. In this way, the cells in the first could be used for studies (e.g., ex vivo expansion), while the majority of the grafts contained in the larger compartment remains un-manipulated and fully viable. Subsequently the two cell populations could be infused in to the patient when the procedures (expansion or other) were completed. This method is refereed to as "Processing Method 3".

In the period 2002-2005, NCBP campaigned to secure Federal funding for cord blood banking with the objective of establishing a solid set of cord blood banks capable of substantially providing for the needs of US and foreign patients and of attaining financial self sufficiency. The campaign was successful and the Cord Blood Stem Cell Act of 2005 was signed by President Bush in December, 2005. The National Cord Blood Inventory was subsequently developed by HRSA as part of the WC Bill Young Cell Transplantation Program, including a Cord Blood Coordinating Center and a Bone Marrow Coordinating Center and an outcomes database under CIBMTR. HHS (HRSA) also sponsors an Advisory Council on Blood Stem Cell Transplantation.

In invited presentations to the Biological Response Modifiers Advisory Committee of the FDA (2003 and 2007), NCBP updated the outcome data derived from the increased numbers of NCBP grafts. In 2007, we showed evidence of significant improvements in the clinical outcomes of patients receiving these grafts, in the context of therapeutic and technological developments in the area, such as the development of an automated controlled-rate freezing/storage/documentation device (ThermoGenesis' BioArchive). We also demonstrated the initial studies on HLA matching and CB grafts, by showing improved outcomes of mismatched transplants where the donor is homozygous for one or two HLA antigens and the recipient is heterozygous for the same antigens. In these cases, survival is as good as in the case of fully (6/6) HLA matched grafts.

An additional technological development was deployed in the period 2005-2007: we started processing the CB units with a device that automated the volume reduction process using functionally closed processing and cryoprotection in disposable bag sets (ThermoGenesis AXP™). The AXP system was validated and adopted by NCBP for routine CBU manufacturing in August of 2006 and is designated here as "Processing Method 4".

It is Method 4 for which NCBP requests the FDA License for the disease indications described in the FDA Guidance. The Application process was initiated six years ago, in 2004. Two pre-BLA meetings were held in 2005 and 2006. However, the application process was interrupted in early 2008, by diverse unrelated reasons, the main one being the mechanism for participation of an independent NCBP in the National Cord Blood Inventory (NCBI), with the contingent need to quickly establish the Distributed Search system, that permits our inventory to be accessible through the federally funded Single Point of Access. This development process was only completed in July of 2009. A second cause of delay was the necessity to move the manufacturing, testing and typing facility to a more appropriate building with the capacity to

allow the GMP processing of up to 50 CBUs per day in a single shift, and increase storage capacity to more than 80,000 CBUs with expansion capability to more than 200,000 CBUs.

Three additional NCBP collection sites became operational in the last years, at Long Island Jewish Hospital in New Hyde Park, NY (2005), Albert Einstein-Montefiore Hospital in Bronx, NY (2006) and after more than 10 years, again at Mt Sinai Hospital in Manhattan, NY (2007). Lastly, a new collection site was added very recently at DeKalb Medical Center in Altanta, Georgia.

NCBP has continued to evaluate clinical outcomes and use the results to improve the selection process of CB units for transplantation. Further, NCBP has pursued collaborative studies with CIBMTR (Center for International Blood and Marrow Transplant Research) and other institutions. These have resulted in several important publications that demonstrated the effectiveness of partially HLA-A, -B and DR matched cord blood transplants in adults[8-10] and children [8, 11, 12], and comparison studies with matched and mismatched adult donor transplants (bone marrow and peripheral blood mobilized stem cell grafts). These will be discussed later in this book.

As of 6/30/11 NCBP has distributed 4380 CBUs for 3982 unrelated transplants in recipients worldwide. The NCBP "Map of Transplants" is attached.

In the following sections of this book, we present:

- a brief description of the new **NCBP facility**, where the Program relocated in May 2009,
- the current methods of collecting, processing, testing, storing, selecting and shipping cord blood products are described in the section **Methods of Manufacturing,**
- the **Outcomes and Safety data of the CB units manufactured by the NCBP** that have been transplanted into unrelated recipients.
- a list of the relevant **NCBP Publications**.

1.1 TABLE: NCBP Processing methods and units in search/transplanted

Manufacturing Method	1		2		3		4	
Time period	**1993 - 1995**		**1995 - 05/1999**		**05/1999 - 08/2006**		**08/2006 - 08/2011**	
Number of Processed CBU	2849		5938		30012		32020	
Number of CBU available in Search (as of 08/2011)	1479		3636		24415		27519	
Number of transplanted CBU	465		902		2240		557	
Number of Transplants	463		887		1981		481	
Number of Patients	448		866		1935		467	
CBU Pre-cryopreservation TNC Median ($x10^6$)	1049		1252		1566		1610	
Pre-cryopreservation TNC/kg Median ($x10^6$)	35.8		40.2		37.6		31.6	
Storage Time* **(N, %)**								
< 1 year	18	(4%)	139	(15%)	297	(13%)	145	(26%)
1 - 5 years	332	(71%)	536	(59%)	1447	(65%)	412	(74%)
5 - 10 years	94	(20%)	167	(19%)	486	(22%)	0	(0%)
10 - 15 years	20	(4%)	59	(7%)	10	(0%)	0	(0%)
> 15 years	1	(0%)	1	(0%)	0	(0%)	0	(0%)
median time in storage (years)	*3.64*		*2.80*		*2.88*		*1.59*	

Number of CBU is higher than the number of transplants reflecting transplants with double unit grafts.

Number of patients is smaller than the number of transplants, because some patients received two transplants.

*Storage time: time from processing to infusion, indicating the period that the CBU has been cryopreserved ("age" of the CB unit).

1.2 ATTACHMENT NCBP "Map of Transplants"

1.3 References

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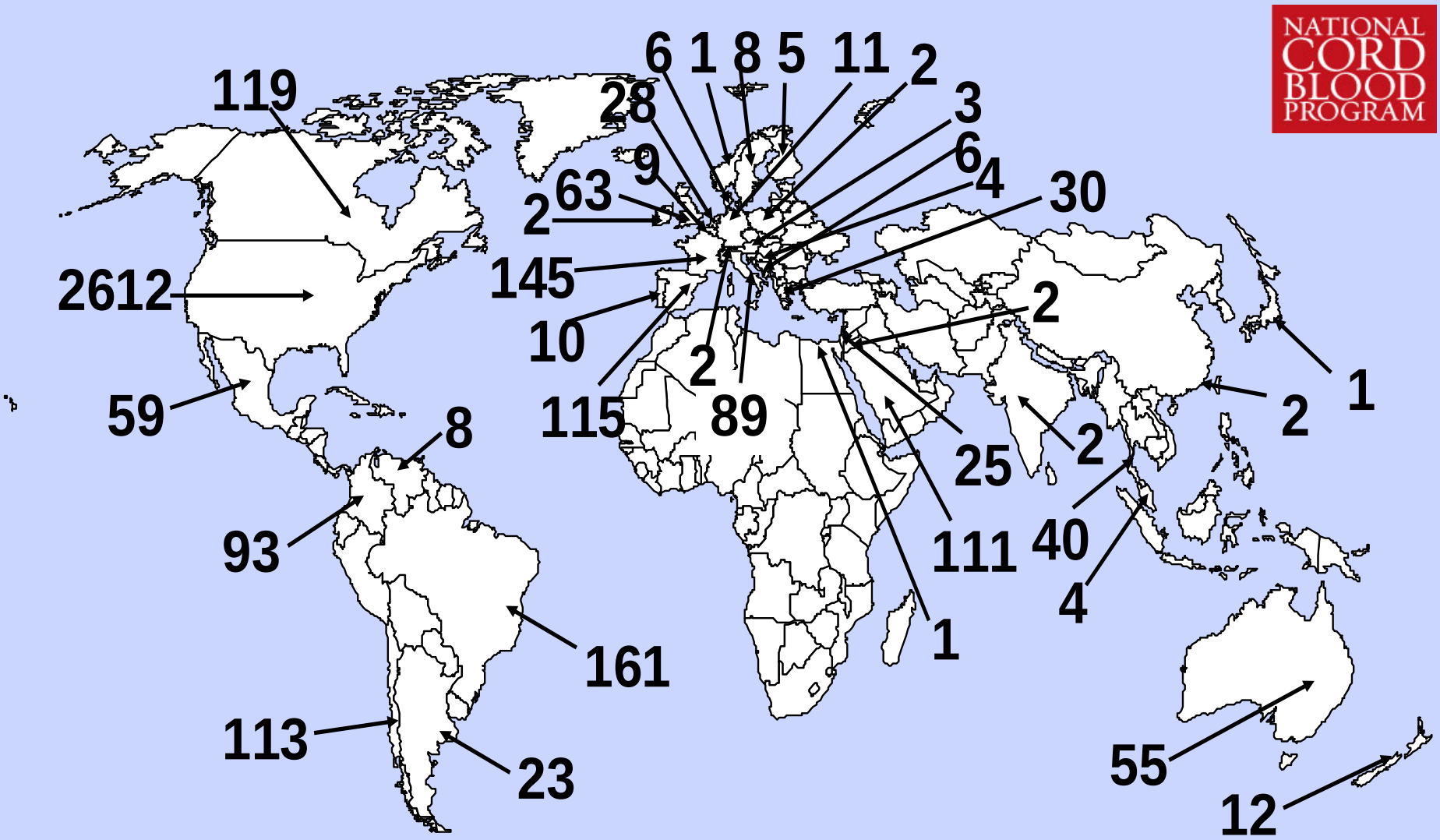

***NYBC* National Cord Blood Program (8/24/93 – 6/30/11):**

4,380 CBUs Shipped for 3,982 Transplants

DESCRIPTION OF THE FACILITY

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1.0 FACILITY DESCRIPTION

1.1 Introduction

The New York Blood Center (NYBC) National Cord Blood Program (NCBP) was founded in 1993, and located at the New York Blood Center's facilities at 310 East 67th Street in New York. The NCBP relocated to new facilities in Long Island City, NY in May of 2009.

The new facility was designed to support the following criteria:

- Accept 50 cord blood units (CBU) per day (13,000 CBU/year @ 5 days/week single shift)
- Distribute up to 1000 CBU per year
- Store 80,000 units of cord blood
- Segregate GMP Processing Area from Quality Control Labs and other areas
- Provide environmental control, segregation and containment to meet cGMP guidelines
- Provide adequate space and layout to match cord blood process and sample flow
- To be flexible, cleanable, and provide ease of maintenance with minimum material handling

The NCBP currently processes approximately 4000 units of cord blood per year and currently stores over 50,000 units.

1.2 General Information

The New York Blood Center's (NYBC's) National Cord Blood Program (NCBP) facilities are currently located at 45-01 Vernon Blvd, Long Island City, NY, 11101.

The total usable building area is a single storey structure with approximately 75,000 square feet, with approximately 20,000 square feet allocated for National Cord Blood Program activities. The remainder of the space contains NYBC's primary blood receiving, processing, testing, and distribution facilities, and includes support facilities such as quality assurance, training, warehouse, maintenance, and administrative offices. The facility contains no research laboratories or activities.

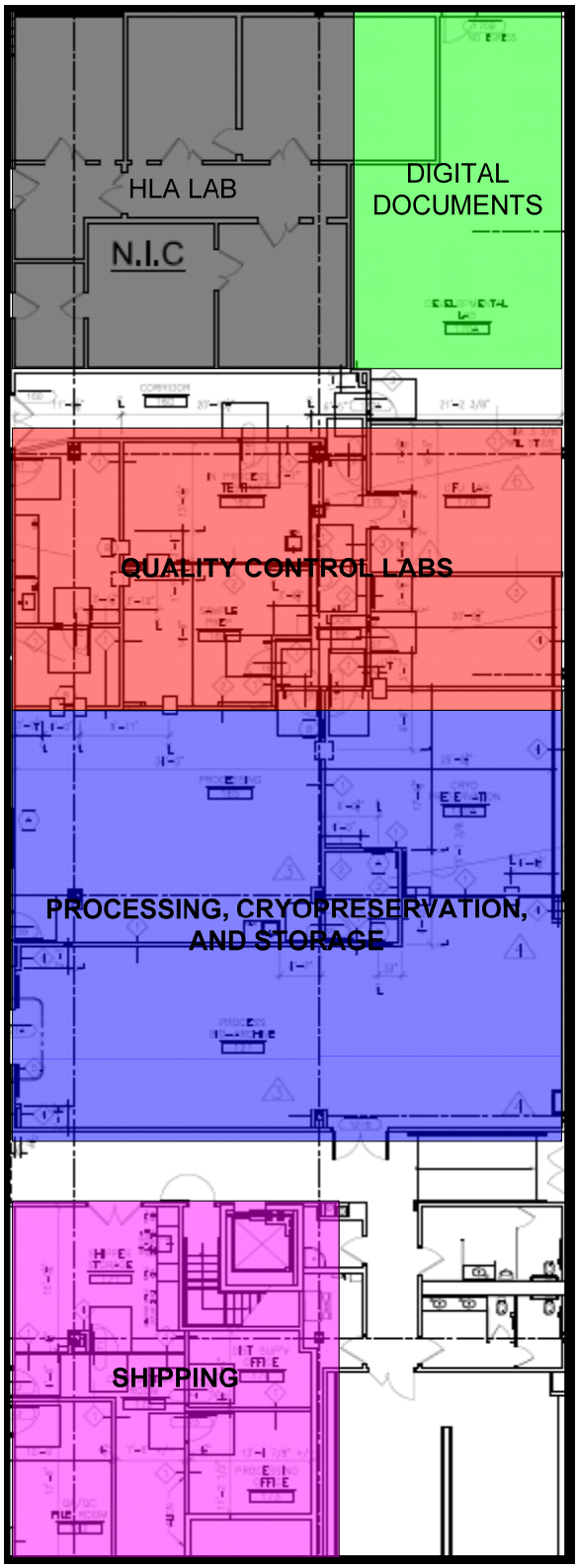


Figure 1 - General Floor Diagram

1.3 NCBP Facility Design Philosophy

In order to maintain segregation of the process areas, the NCBP facility has been designed with containment zones. Rooms have been sized to be efficient and to optimize cleaning and/or sanitization of spaces. A single large work area is able to be divided into smaller zones to minimize the potential of contamination. An airlock is provided in order to separate rooms and areas from each other, and where required, to mitigate the risk of contamination. Corridors have been designed to facilitate travel between clean spaces, technical spaces and office spaces. Where possible, windows are used on corridors to allow observation of equipment, processes and people without entering GMP Process Areas.

The general floor diagram of the NCBP facilities is shown in Figure 1. The HLA Lab and Digital Document rooms (shaded green) are located in the northern most portion of the area and are accessed through card access controlled doors via general circulation corridors.

The Quality Control Labs (shaded red) include Sample Prep, In Process Testing, CFU Lab and Plasma Process Rooms, and are located adjacent and to the north of the Processing, Cryopreservation, and Freezing/Storage Areas (shaded blue).

A dedicated Cord Blood Shipping area (shaded pink) is located in the southernmost portion of the facility, and includes shipping coordination, dry shipper storage, and LN2 filling station.

Additional NCBP dedicated areas include:

- Cord Blood Receiving/Accessioning
- Flow Cytometry Lab
- Administration, Medical, Quality Unit Offices
- Warehousing, Shipping, Receiving
- Mechanical Freezer Retention Sample Storage Room

A detailed floor plan of the processing and testing labs is attached, Drawing Number A1-0, Cord Blood Facility.

1.4 Floor Diagram of Processing Area

The GMP Process Area, shown in Drawing Number A1-1, GMP Process Area (attached), is segregated into a self-contained module that serves to protect the integrity of the different environments as well as protect the products from the testing activities and from incoming raw materials. The cGMP Process area includes:

- Gowning Room
- Processing and Supervisor's Office
- CryoPreservation
- Quality Control Lab – Plasma Processing
- Quality Control Lab – Sample Prep

2.0 PRODUCT, SAMPLE, PERSONNEL AND WASTE FLOWS

Personnel entry into the GMP Processing Area is via airlock, while Cord Blood product is received via pass-through window. Both Cord Blood Product and samples leave the GMP Process Area via pass-through windows. Cord Blood product flow from room to room is unidirectional. Transfers of Cord Blood product via pass-throughs promotes the unidirectional material flow and segregation from personnel movement. Samples are handled using the same flow philosophy. The designed flow minimizes the opportunity for cross contamination or mix up. Product, Sample and Personnel flows are shown on Drawing A1-2. Waste Flows are shown on Drawing A1-3.

3.0 HEATING, VENTILATION, AND AIR CONDITIONING (HVAC)

The HVAC system serving the National Cord Blood Program (NCBP) GMP Process Area is designed to provide dedicated, filtered, pressurized and controlled temperature/RH air, with high air changes per hour. The system makes use of two (2) dedicated air handlers, Air Handling Unit (AHU) 2 and AHU 2A. AHU 2 provides 100% outside makeup air to AHU 2A, as well as supply to adjacent non GMP Process areas. AHU 2A provides dedicated air to the GMP Process Area. Refer to Drawing H1.1, AHU Zoning Diagram, and Drawing H1-0, Classification and Pressurization.

4.0 PROCEDURAL CONTROLS AND FACILITY/EQUIPMENT DESIGN

The Facility/Equipment Design and the Procedural Controls were developed in conjunction to prevent contamination, cross-contamination, and mix ups, and to promote an aseptic process with controlled, consistent, and high quality results.

Table 1 provides a listing of these Procedural and Facility/Equipment Design Controls.

Table 1: Contamination Precautions

Procedural Controls	Facility/Equipment Design Controls
Access Control Procedure	Location within Facility
Gowning Procedure	Environment Controls
Cleaning procedure	GMP Area Adjacencies
Environmental Monitoring Procedures	Flow Controls
Automation	Lab Layout
One-At-A-Time	Closed Process

4.1 Facility Design Controls

- Location within Facility: The NCBP is located in the rear of the LIC building, with the entrance to the GMP Process Area physically separated from the main entrance/exit of the facility, the warehouse, and the loading docks.
- Environment Controls: The GMP Process Area includes a dedicated air handler with 100% fresh air make-up (no recirculation), 95% pre-filters, terminal High Efficiency Particulate Air (HEPA) filters, and 20-40 Air Changes Per Hour. The containment between rooms is achieved by airlock, pressure differences between rooms, and airflow toward the point of greatest need for containment or away from the area requiring the greatest level of segregation or cleanliness.
- GMP Area Adjacencies: The room adjacencies within the GMP Process Area were designed to support uni-directional and minimum distance flow of materials, product and personnel.
- Flow Controls: Transfers from functional areas are via pass-through windows to promote uni-directional material flow and segregation from personnel movement. The pass through windows serve as airlocks to ensure containment between rooms, and also to facilitate tracking of all product and samples via dedicated barcode tracking system. Personnel entry and exit from the GMP Processing Area is bidirectional but via a shared airlock where gowning and degowning takes place. The airlock further enhances segregation of function between adjacent environments. The cord blood receiving and accessioning personnel do not have or need direct access to the cord blood processing area. The BioArchive Storage area personnel do not have or need direct access to the Cryopreservation area. Waste is handled after normal working hours when processing is complete.
- Lab Layout: Each lab area is designed to promote good technique and procedure by providing adequate and dedicated space for each unit operation and equipment.
- Closed Process: The processing method, using the automated AutoXpressTM (AXP) platform and bag set is a functionally closed process.

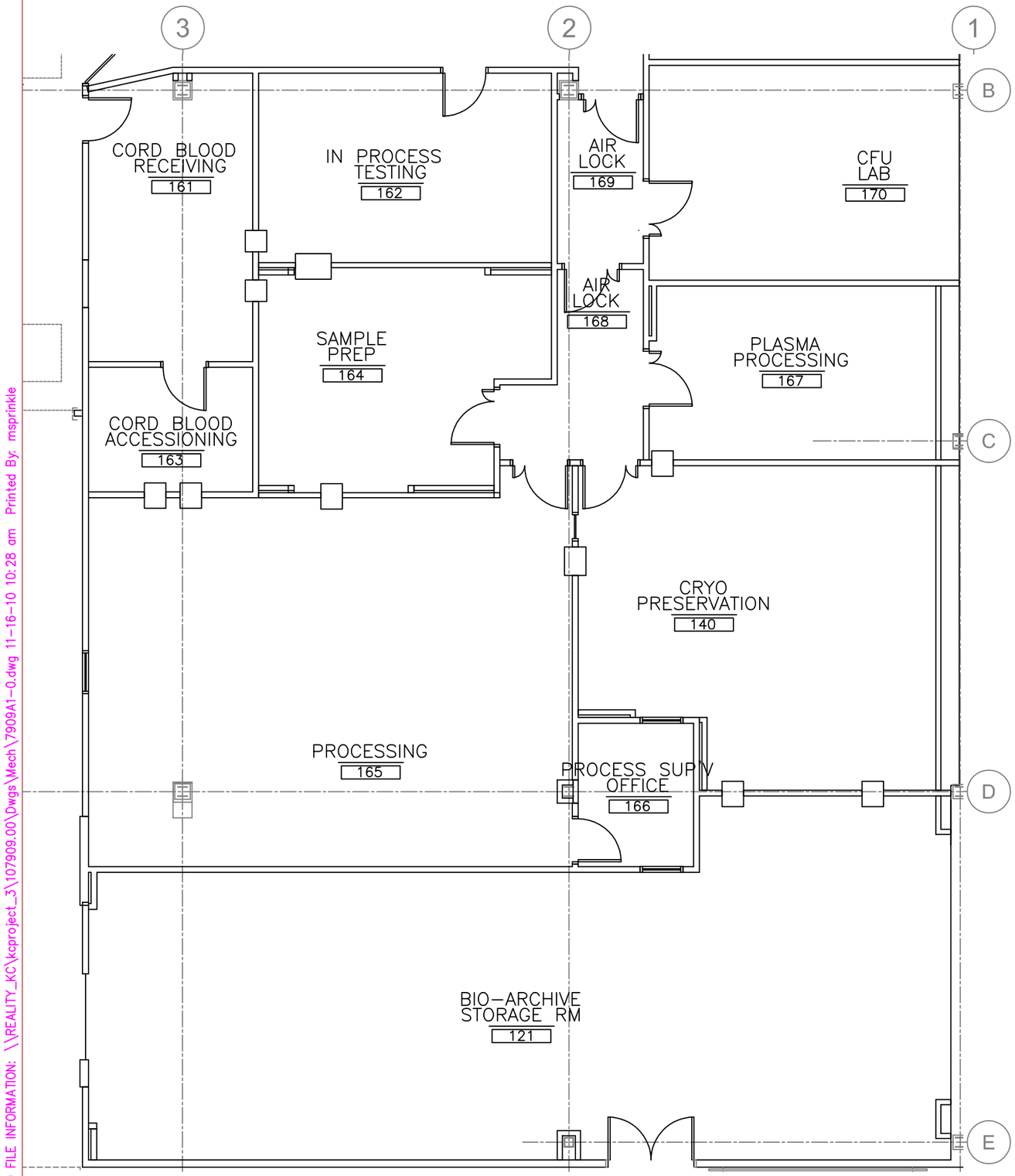
4.2 Operational Controls

- Access Control Procedure: Access to the GMP Process Area is restricted to those personnel trained in the appropriate procedures and who perform required functions in these areas. Access is controlled by use of card readers.
- Gowning Procedure: All personnel entering the GMP Process Area are required to gown in accordance with approved procedures, to promote control of the environment and decrease the chance of contamination.
- Cleaning Procedure: The GMP Process Area has a dedicated cleaning procedure. The cleaning procedure includes rotation of cleaning agents designed to prevent selection for resistant organisms.

- Environmental Monitoring Procedure: The rooms and equipment (refrigerators and freezers) are monitored on a daily basis for temperature, as well as relative humidity where required. In addition, the GMP Process Area is monitored on a quarterly basis for airborne non-viable particulates, and airborne and surface viables.
- Automation: The process includes extensive use of automation, including barcode scanning. and specific software applications designed to control the flow of information into the database.
- One-At-A-Time: The NCBP requires staff to use the one-at-a-time philosophy of product and sample handling, to reduce the chance of mix ups, erroneous identification and cross contamination.

4.3 In Process Controls

- Each unit of Cord Blood is sampled and tested for contamination.



NATIONAL CORD BLOOD PROGRAM

New York Blood Center

45-01 Vernon Blvd, 45
Long Island City, NY 11101-5201

TITLE:

FLOOR PLAN
CORD BLOOD
FACILITY

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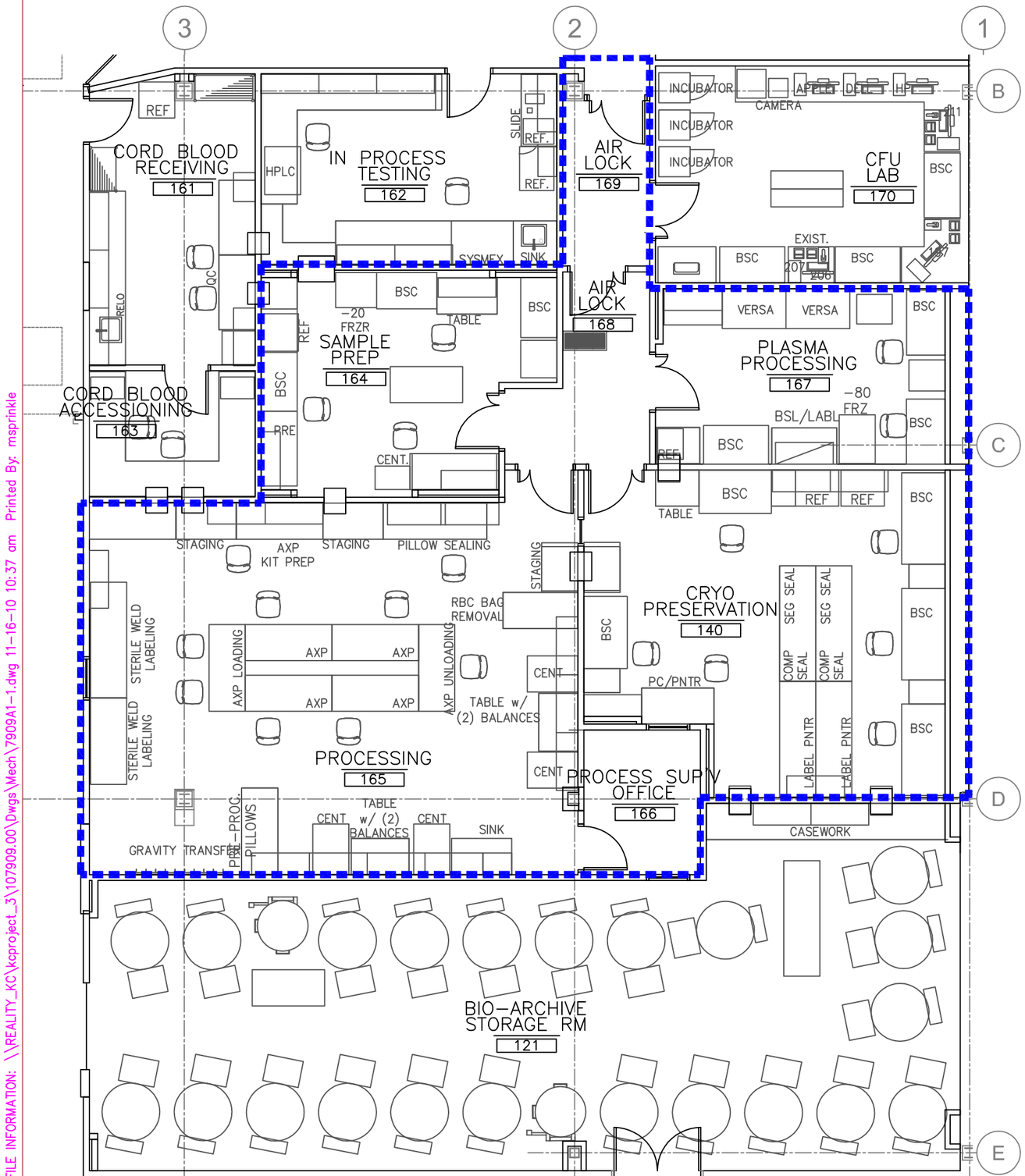

LEGEND

GMP PROCESS AREA

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

45-01 Vernon Blvd, 45
Long Island City, NY 11101-5201

TITLE:

FLOOR PLAN
GMP PROCESS
AREA

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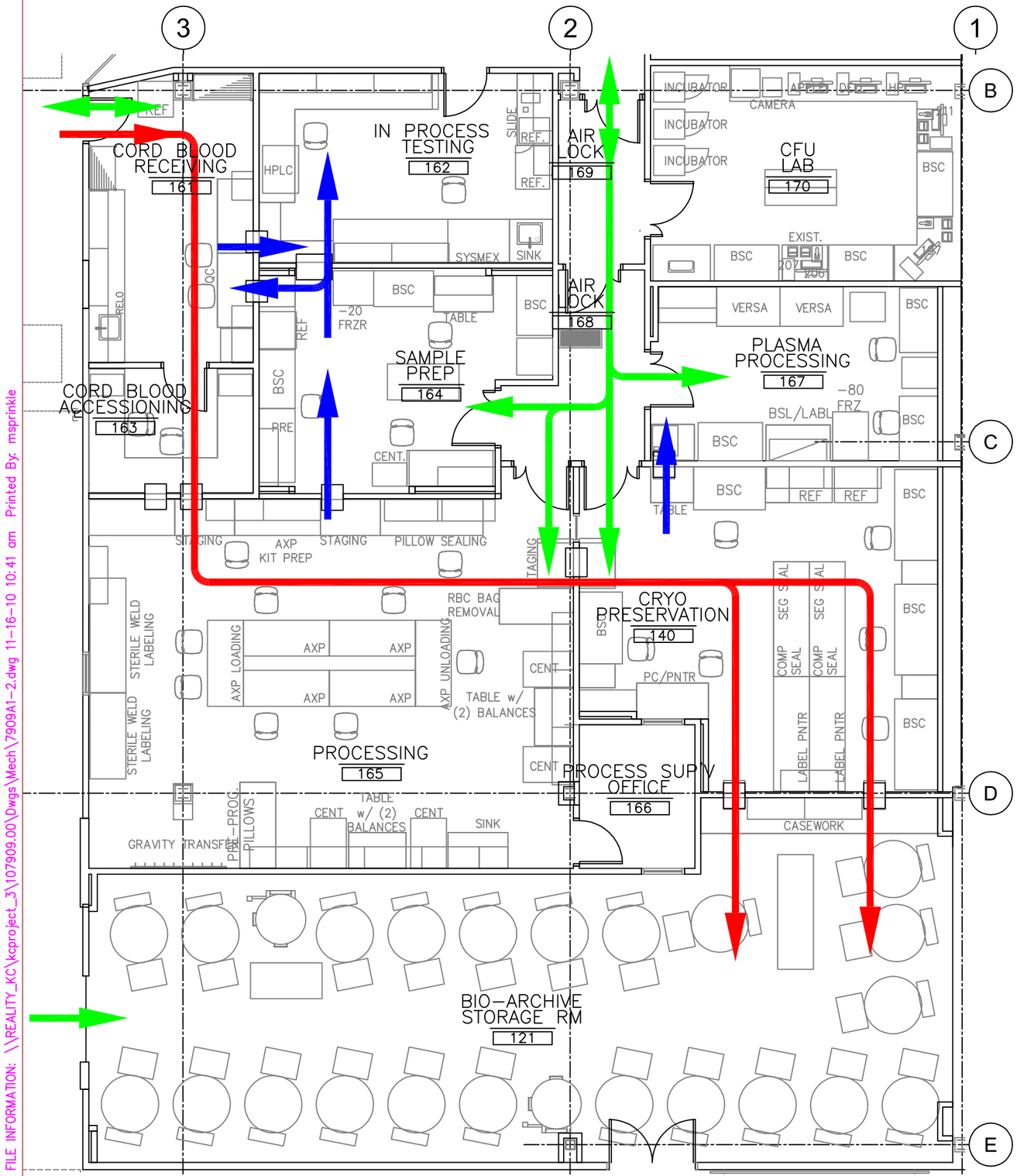

LEGEND

PRODUCT FLOW

SAMPLES FLOW

PERSONNEL FLOW

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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TITLE:

FLOOR PLAN
PRODUCT, SAMPLE
& PERSONNEL FLOW

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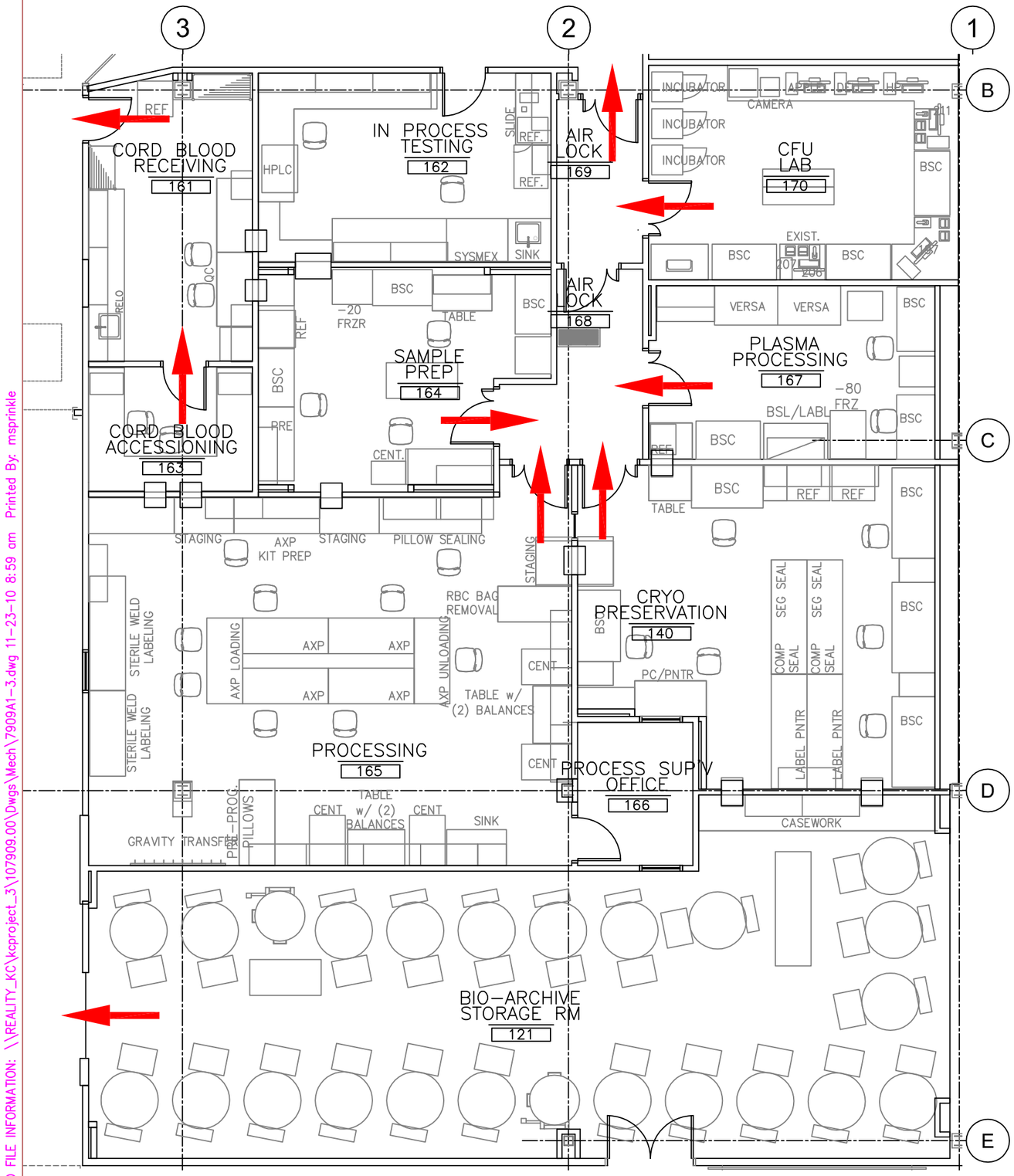

LEGEND

WASTE FLOW

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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Long Island City, NY 11101-5201

TITLE:

PROCESS
FIRST FLOOR PLAN
WASTE FLOW

DWG. NO:	REV.
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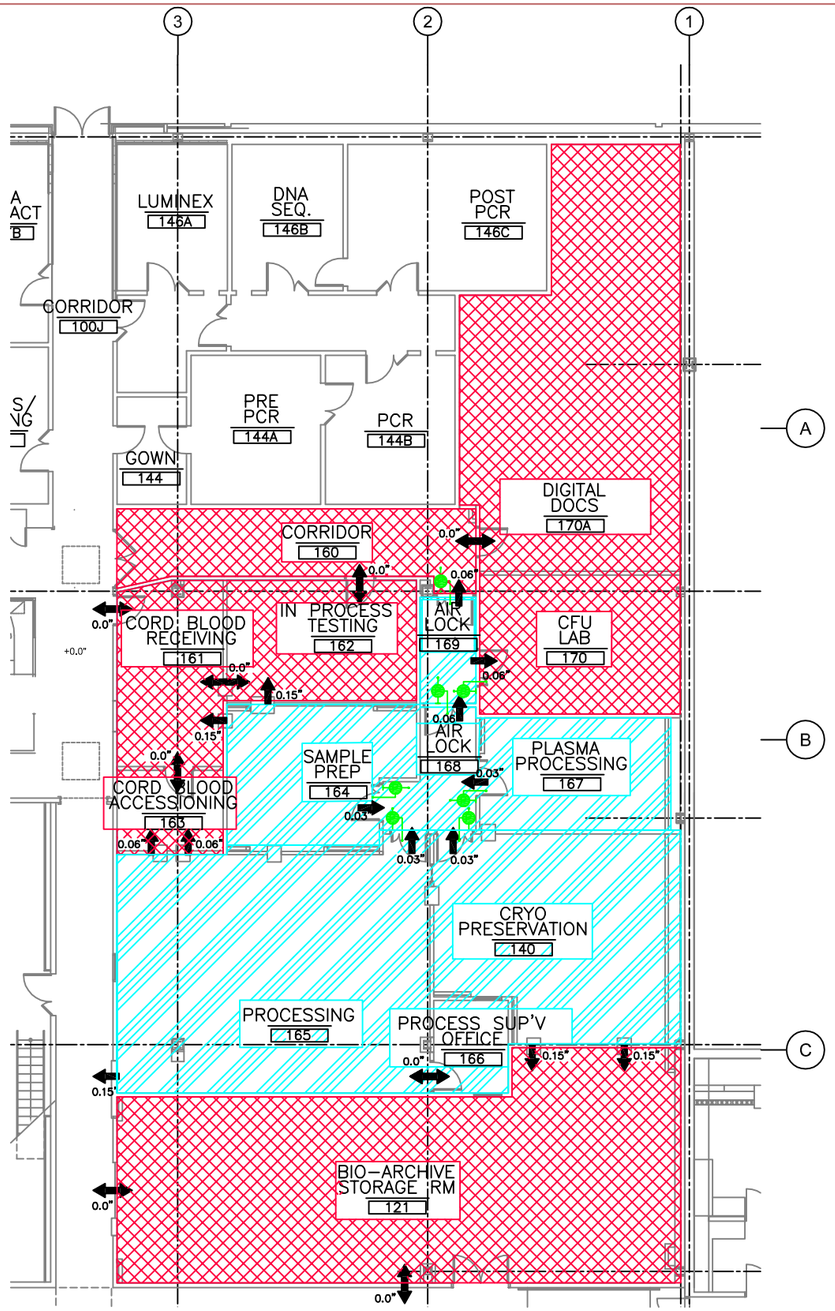

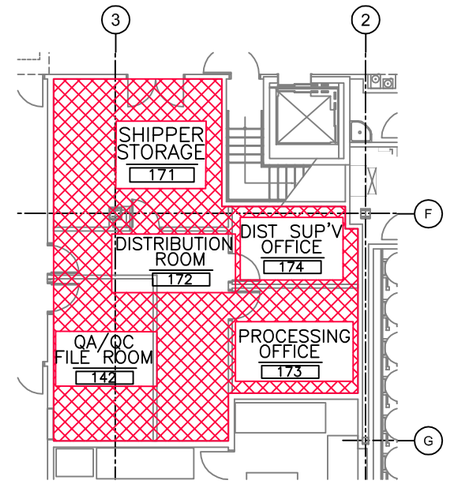

CLASSIFICATION LEGEND

OPERATION: CONTROLLED / UNCLASSIFIED

OPERATION: UNCLASSIFIED

+0.12 NOMINAL RELATIVE ROOM PRESSURE TO EXTERIOR (DESIGN)

0.06" NOMINAL PRESSURIZATION AIRFLOW WITH DIFFERENTIAL PRESSURE (DESIGNED)

WALL-MOUNTED ROOM DIFFERENTIAL PRESSURE GAUGE

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

45-01 Vernon Blvd, 45
Long Island City, NY 11101-5201

TITLE:

HVAC CLASSIFICATION & PRESSURIZATION

DWG. NO:	REV.
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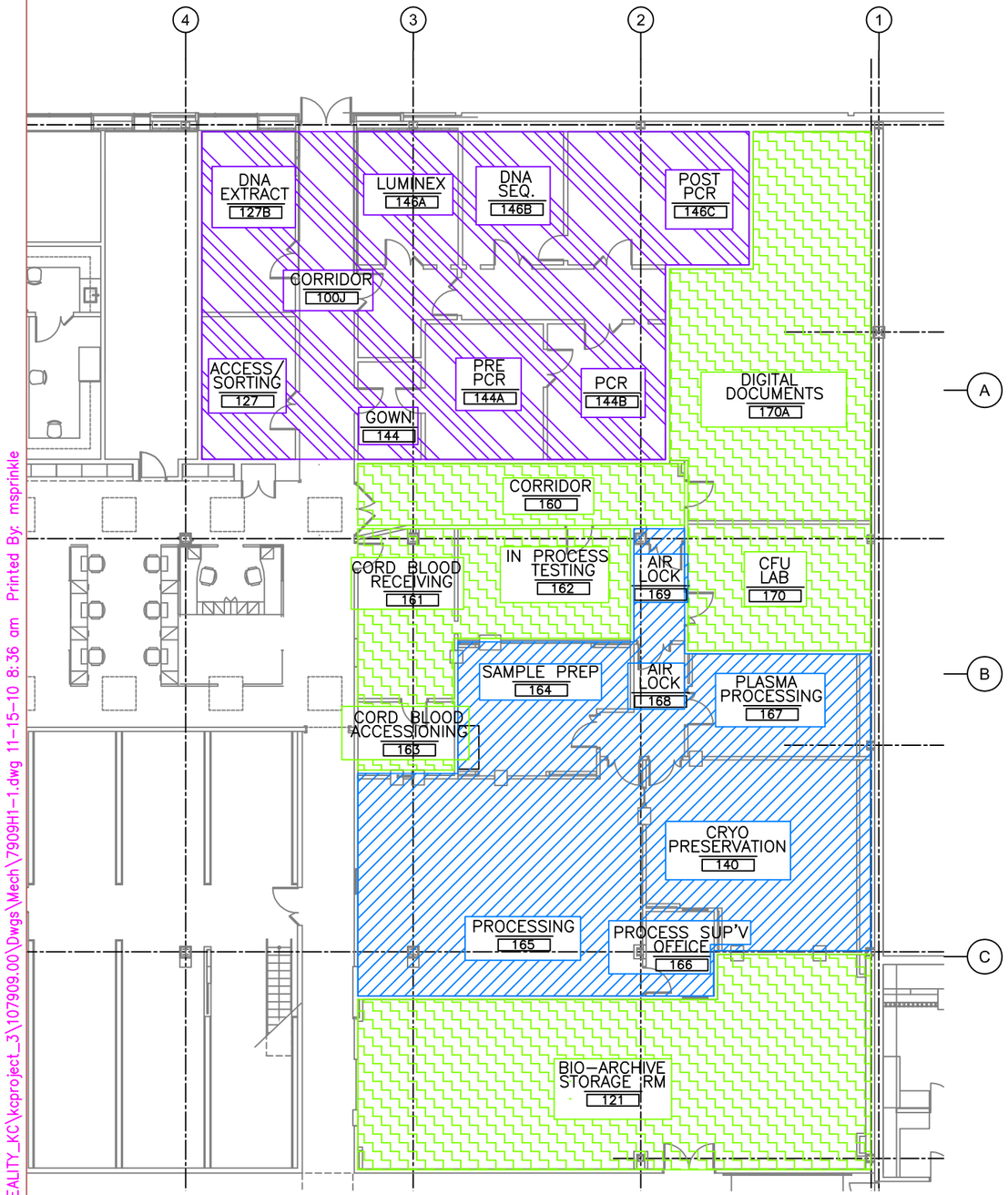

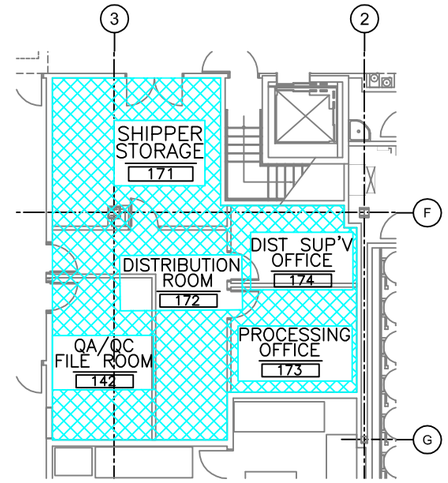

AHU LEGEND

- AHU-1
- AHU-2A
- AHU-2
- AHU-3

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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TITLE:

HVAC AHU ZONING DIAGRAM

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DESCRIPTION OF MANUFACTURING

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DESCRIPTION OF MANUFACTURING

The following section is a brief description of the Operations of the National Cord Blood Program (NCBP), including systems supporting the Operations. The section is accompanied by four summary-level flow diagrams (W1-1: Collections; W1-2 : Processing; D1-1 : WebSearch (Access/Search/Reservation/Shipment of CB units), CS1-1: Computer Systems Architecture). Numbers in the text [e.g. (1 - W1.1] correspond to steps in the respective flow diagrams.

1.1. Cord Blood Collections

1.1.1. Collection sites

NCBP has contracts with eight Hospitals for collection of cord blood (CB). These are: North Shore Hospital (Long Island, NY), Long Island Jewish Medical Center (Long island, NY), New York Presbyterian Hospital (Manhattan, NY), Montefiore Medical Center/Albert Einstein College of Medicine of Yeshiva University Weiler Hospital (Bronx, NY), Mt. Sinai Hospital (Manhattan, NY), INOVA Fairfax Hospital (Fairfax, VA), University Hospitals of Cleveland (Cleveland, OH) and DeKalb Medical Center (Atlanta, GA). These contracts include use of space and reimbursement of costs borne by the Hospitals only, and do not constitute contract manufacturing arrangements. All collections staff report directly to and are under the control of the NCBP.

1.1.2. Informed consent and collection process

NCBP staff at each participating collection site is responsible for obtaining maternal consent, collecting CB units and performing review of medical records of the mother and newborn.

Program Staff (mostly Registered Nurses) obtain “Maternal Permission to Collect and Review of medical records” from mothers admitted for delivery (1 – W1-1). NCBP staff members attempt collection on every birth during their shift, unless any of the following is recorded in the medical records (2 – W1-1):

- The mother has a blood transmissible infection, (e.g. Human Immunodeficiency Virus, Human T Lymphotropic Virus, Hepatitis B or C Viruses or syphilis) or sepsis
- There is a known genetic disease that could be transferred to the recipient by transplantation
- Pregnancy is a result of egg donation, or sperm donation from a donor other than the spouse (so that family history is not available)
- Mother has Creutzfeldt Jacob Disease (CJD) or has a blood relative with vCJD
- Mother had cancer or leukemia (at any time in her life), or received chemotherapy or immunosuppressive drugs during this pregnancy
- Mother had toxoplasmosis during this pregnancy
- Mother had sex with an HIV+ person during pregnancy
- Mother has not given permission to collect the CB unit

- The placenta or the cord have been damaged potentially allowing microbial contamination or cross contamination
- Parents have arranged for private cord blood storage

Collection of the cord blood is performed ex utero, from the delivered placenta (3 – W1-1) at a dedicated collection laboratory located in the hospital's labor and delivery area (4 - W1-1).

The surface of the cord is cleaned to remove maternal blood and possible infectious agents, facilitating an aseptic collection procedure (5 - W1-1). The needle of the collection bag (with CPD-A anticoagulant) is inserted into the umbilical vein of the cord (6 - W1-1) and the cord blood drained into the collection bag by gravity (7 - W1-1).

After the collection is complete, the collector weighs the bag (8 - W1-1) and performs a Total Nucleated Cell ("TNC") count on the unit (9 - W1-1) using an automated hematology analyzer in the collection laboratory, to determine whether the collected cord blood unit meets the current minimal TNC requirement for processing and storage (≥ 140.0 x 107 "clinical unit") by the NCBP.

If the cord blood collection meets NCBP minimal cell content requirement, the staff member who collected it, affixes a preprinted label with a visual – and machine (bar coded) - readable ID number to the unit and to all associated specimen tubes and document pages (10 - W1-1).

The NCBP nurse approaches the mother (after she has rested) and (11 - W1-1)

- Obtains written informed consent for donation to the NCBP and for all other required donation procedures[1]
- Conducts a personal and family history and risk factor interview with the mother (and, if available, the father)
- Performs/documents a review of the mother's medical record
- Performs/documents a review of the infant's medical record
- A blood specimen is collected from the mother for infectious disease testing and HLA typing.

Alternatively, the mother is informed by a staff member, that the collected cord blood unit does not meet the current TNC NCBP requirement.

1.1.3. Collection Site Storage, Packing and Transportation of Collected CB units:

CB units are kept at room temperature in the collection site laboratory (12 - W1-1).

CB units are packed in absorbent material within individual plastic bags and shipped in OSHA-compliant packing boxes that maintain a temperature range of 2°C to 30°C (13 - W1-1). The packing box includes a thermocouple-based electronic temperature monitor that records the temperature from the time of packing until receipt by the NCBP Processing Laboratory.

[1] Consent must be obtained for the unit to be eligible to become part of the NCBP inventory. If the mother refuses consent for banking the unit for transplantation, or for research, the collected cord blood is discarded, in accordance with the mother's wishes as recorded on the informed consent form.

1.2. Cord Blood Processing

The NCBP Processing facility is located in Long Island City, NY and it is the only location at which the NCBP processes and stores cord blood units, and performs all other activities described in the following sections.

1.2.1. Accessioning

When the units are received at NYBC NCBP's Receiving Area (1 – W1-2) the bags and documentation are inspected (2 - W1-2).

The Hospital shipping boxes are unpacked and the temperature data loggers are checked. A record is created for the unit in the NCBP database by scanning the unique bar coded unit ID number. The accessioning date and time are assigned by the computer.

The CB unit is weighed on an electronic balance and the weight is transferred electronically to the database. The computer calculates the blood volumes (total with anti-coagulant, collected and residual) based on the unit weight, dry bag weight, anti-coagulant volume and the volume of samples to be taken for pre-process testing.

The computer also generates a set of visually-readable and bar-coded ID labels for the respective QC worksheets. One of these labels is used to identify the newly accessioned CB unit.

1.2.2. Processing of CB units

Processing is performed with the AutoXpress™ (AXP) automated, closed processing System (3 - W1-2) [AXP AutoXpress™ Platform and the AXP single-use processing kit - 510 (k) (BK 070006)]. The procedure reduces the volume of the CB unit by removing plasma and the bulk of the red blood cells.

The cord blood collection bag is sterile-welded to the AXP bag-set, the bags and the sampling pouches (pillows) of the AXP bag-set are labeled and the blood transferred to the "Processing Bag" of the bag-set through a clot filter (4 - W1-2). The bag-set is mixed by turning end-over-end and a ~7 mL aliquot of well-mixed cord blood ("pre-processing sample") is aspirated into an integral plastic sample pouch. The specimen pouch is then separated from the bag set by sealing the connecting tubing, and is sent for testing (5 - W1-2).

During centrifugation (6 - W1-2) the AXP device sequentially separates the bulk of the red blood cells (RBC) into the RBC bag (7 - W1-2); the buffy coat (~21 mL) into the two-compartment freezing bag (8 - W1-2); the excess plasma remains into the processing bag (9 - W1-2). The AXP device processing data is automatically downloaded into the AXP database using the XpresTRAK™ software and the processing report is printed and reviewed.

A smaller sampling pouch (~0.5 mL sample) connected to the freezing bag tubing, is used to obtain the post-processing samples for testing (10 - W1-2). The RBC bags are transferred to the QC lab to be used by the HLA lab for DNA extraction and typing.

The bag-set with the freezing and plasma bag still attached is then transferred through a pass-through window into the Cryopreservation lab.

1.2.3. Cryoprotection, Controlled-rate Freezing and Storage in the BioArchive™ system

Addition of the cryoprotectant takes place in a biological safety cabinet (11 - W1-2).

Five mL of sterile, refrigerated 50% (v/v) DMSO and 5% (w/v) Dextran 40 cryoprotectant solution from a single-use vial is slowly added with a syringe pump to the freezing bag containing the volume-reduced unit (to yield a final concentration of 10% DMSO and 1% Dextran 40) through an integral, sterile, 0.2µ filter (11 - W1-2).

The CB unit is kept cold with gel packs and continuously mixed during the addition of DMSO/Dextran. The freezing bag inlet tubing is filled to a specified length with product and sealed at the far end of the length, allowing the separation of the plasma bag from the freezing bag; three segments are created in the freezing bag's tubing by additional seals to the tubing (12 - W1-2). The bag bridges connecting the two bag compartments are also heat sealed.

The plasma bag is transferred to the QC lab where a portion of the plasma is removed via a sampling port for sterility testing (9 - W1-2).

The freezing bag is overwrapped in a Teflon bag (13 - W1-2) and the overwrap bag is sealed.

The overwrapped freezing bag is placed into a stainless-steel canister, labeled with the same ID number as the CB unit, transferred through a pass-through window into the BioArchive storage room and inserted in a Control-Rate-Freezing (CRF) module for controlled-rate freezing in the BioArchive System (14 - W1.2). The BioArchive System (Master File BB-MF-12835) is a hardware/software system used for the controlled-rate freezing, liquid nitrogen storage and retrieval of CB units, with continuous monitoring and documentation.

Transfer of CB units from Accessioning, to Processing, Cryopreservation Laboratories and the BioArchive Storage room takes place through pass-through windows. Similarly, transfer of the RBC bag, plasma bag and the pre- and post-processing samples to the QC Laboratory for testing, also takes place through pass-through windows.

1.2.4. Storage aliquots

Aliquots of plasma, viable cells and DNA from CB units and maternal blood samples are separated and preserved for future testing.

1.2.5. Labeling

All aliquots for testing and storage, all bags and work-sheets are labeled with the unit's unique ID number (visually-readable and bar coded). Additional labels are generated by scanning the original label to print "copy-cat" adhesive labels.

1.3. Cord Blood Testing

The following tests are performed and results are entered into the NCBP database. Data are transferred electronically to the database from the testing equipment and testing laboratories (Exception: special testing: reports are reviewed and results are entered manually).

Cord Blood Testing

Test	Performed by	Location	Comments
HLA-A, -B, -DR	NYBC HLA Laboratory	NYBC	NYS DOH Licensed ASHI, CLIA-Accredited
ABO/Rh	Creative Testing Solutions (CTS)	Tempe, AZ	NYS DOH Licensed
CBC	NCBP QC Lab	NYBC	NYS DOH Licensed
CD34+, CD45+ cell counts & viability	Flow Cytometry Laboratory	NYBC	NYS DOH Licensed
CFU assay	NCBP QC Lab	NYBC	
Microbiology-screening	NCBP QC Laboratory	NYBC	NYS DOH Licensed
Microbiology-organism identification	Beth Israel Microbiology Laboratory	New York, NY	NYS DOH Licensed
Infectious Disease Markers*	Creative Testing Solutions (CTS)	Tempe, AZ	NYS DOH Licensed
Infectious Disease Markers (stored samples-NAT)	Labs, Inc.	Centennial, CO	NYS DOH Licensed
Sickle Hemoglobin, other Hb traits (Hemoglobinopathy screening)	NCBP Processing Lab, screening	NYBC	NYS DOH Licensed
Hemoglobinopathy testing	Hemoglobinopathy Referral Laboratory: Molecular testing Children's Hospital Oakland Research Institute	Oakland CA	NYS DOH Licensed

* Additional selective ID testing (e.g., HBV PCR in stored samples, or testing for toxoplasmosis is provided by Focus Diagnostics Laboratory (Focus Diagnostics, Inc., Cypress, CA).

Note: Although the CFU assay is not a FDA required test, NCBP has a new Laboratory and has implemented a CFU methodology that combines the traditional assay with high resolution digital imaging so that images can be stored and reviewed at any point in the future, form CBU samples prior to cryopreservation, as well as segments thawed after cryopreservation. Further, evaluation is completed for an automated CFU counting system, so that this assay becomes standardized.

1.4. Access to Inventory, Search, Reservation, and Shipping of CB units

1.4.1. Search

Transplant centers or registries submit search requests to identify units in the NCBP inventory that match the patient's HLA type (tissue typing). These search requests can be made directly to NCBP through Fax, through NCBP's WebSearch (the online NCBP access for search and acquisition of CB units) application (1 – D1-1), or through the Single Point of Access of the National Cord Blood Inventory (NCBI). Although some details differ, the essential steps are similar.

A preliminary match report is provided to the transplant center or registry with all matching units, listed by number of matched antigens at the HLA-A, -B, -DRB1 loci (A and B antigens at serological resolution, DRB1 alleles at the DNA sequence level), i.e., matched at 6/6, 5/6, 4/6 loci and, within each level, by TNC content. When the transplant center expresses interest in a unit, detailed information on the CB unit is provided for review (5 – D1-1).

1.4.2. Reservation

When a CB unit is identified for possible transplantation, a reservation is made by the transplant center or registry and the unit is identified as "reserved" in the Search Inventory so that it is no longer available to other patients (2 - D1-1). A reservation initiates confirmatory HLA typing (3 - D1-1), using a cord blood sample from the segment of attached tubing that is integral to the frozen unit. Additional infectious disease testing (e.g., CMV PCR) may also be performed, if indicated (4 - D1-1).

Medical review is performed prior to CB unit release for transplantation (7 - D1-1).

1.4.3. Shipping to transplant centers

Transplant centers request that a CB unit be shipped for receipt on a specific day (6 - D1-1). The unit is normally received before the patient begins conditioning treatment in preparation for transplantation.

After the review of all data, tests and processes has been completed, the product and the accompanying documentation are prepared for shipment (including the instructions for thawing, preparing the product for infusion and emergency product recovery, included in this Briefing Document). The requested unit in its protective stainless steel canister is securely placed in a dry shipper, validated and prepared for shipment with adequate amounts of liquid nitrogen (8 - D1-1). A battery-operated temperature logger monitors the shipment continuously. Shipment is by commercial air couriers, with the shipment tracked by NCBP staff and arrival at the transplant center confirmed. Dry shippers are subsequently returned to the NCBP.

1.4.4. Transplant Patient Follow Up

The NCBP tracks the travel of CB units shipped for transplantation.

If a CB unit is not used upon receipt, NCBP does not permit the unit to be returned to its Inventory, because the unit's continuing integrity and stability cannot be guaranteed.

Transplant centers should report the following to the NCBP (reporting is currently done mostly to the Center for International Blood and Marrow Transplant Registry-CIBMTR, which updates participating cord blood banks periodically)

- CB unit condition upon arrival;
- CB unit thawing results and infusion of the unit
- The status of the patient at the time of transplant
- Infusion adverse events
- The outcome of transplantation

1.5. Systems Supporting Operations

1.5.1. Computer Systems

The NCBP Application Enterprise System (NAES) is an architecture that supports all computer controlled manufacturing steps (across all the functional entities by integrating and coordinating their business process.

At the core of the NAES is a controlled centralized data repository (Oracle Database Management system) which acquires information from and supplies information into the application components. The NAES is designed and implemented as a multi-tier architecture, where application logic is partitioned among various servers and components that are based on the Microsoft and Oracle environment and infrastructure. This application partitioning creates an integrated information structure which enables consistent, secure, fast, and reliable access to the critical data. The NAES also implements strong user authentication and data integrity measures to comply with FDA regulation including HIPAA and 21 CFR Part11 regulations.

A diagram of the Computer Systems Architecture is provided as CS1-1.

1.5.2. Quality Systems Management

The NCBP Quality Function is under the direction of the Senior Vice President of Quality and Regulatory Affairs of New York Blood Center.

QA is responsible for releasing new CB units to the Search Inventory assigning their status as Licensed (after a license is granted) or IND , or rejecting products, reviewing standard operating procedures, deviations and incidents.

Staff members are trained in appropriate cGMPs and cGTPs and receive annual refresher training in these important areas.

1.5.3. Quality Controls and Proficiency Testing Programs:

- SOPs have been developed for all procedures of the NCBP.
- Validations have been performed on all critical aspects of the cord blood manufacturing including collections, processing and cryopreservation, analytical testing methods, storage, shipping and computer applications.

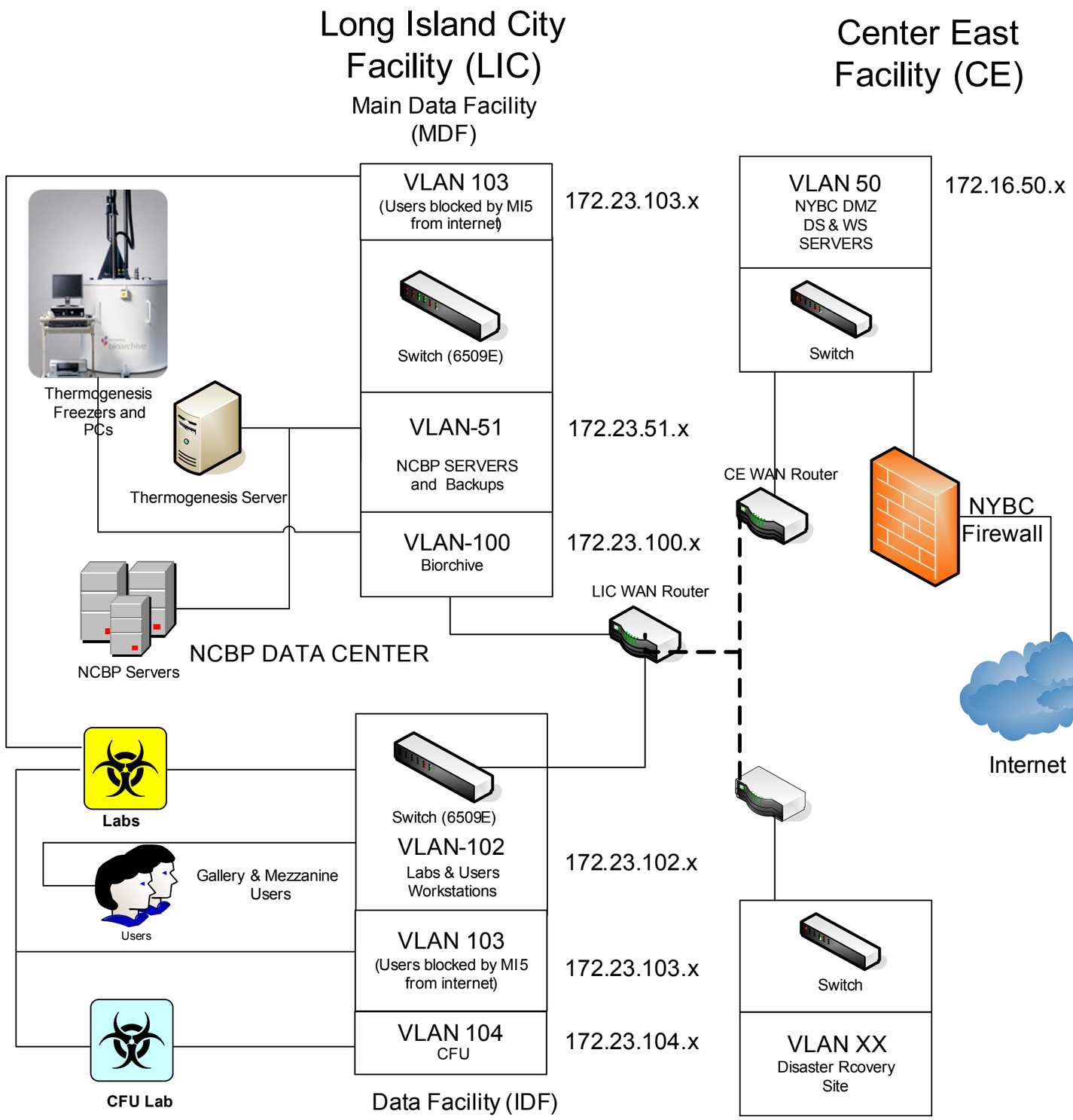
- Testing is performed according to the package inserts of licensed test kits, and NCBP laboratories participate in the New York State Department of Health and/or CLIA and College of American Pathology (CAP) Proficiency programs.
- NYBC maintains a properly constituted Institutional Review Board (IRB), which reviews collection consent forms and the NCBP protocol annually and address issues of donor recruitment confidentiality pursuant to all state and federal regulations.

1.5.4. Personnel training, qualifications and competency

- SOPs and the required training and competency and proficiency testing cover all NCBP areas.
- Staff receives formal training on all SOPs associated with tasks to which they are assigned, current Good Manufacturing Practices ("cGMP") and blood borne pathogens, supplemented by on-the-job training.
- Annual competency review of staff is performed and documented. Annual performance evaluations are required for each NYBC employee ensuring satisfactory performance of tasks and/or a corrective action plan to improve performance.

1.6 ATTACHMENTS: Summary flow diagrams

- W1-1: Collections
- W1-2: Processing
- D1-1: WebSearch (Access/Search/Reservation/Shipment)
- CS1-1: Computer System Architecture



NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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TITLE:
COMPUTER SYSTEM ARCHITECTURE

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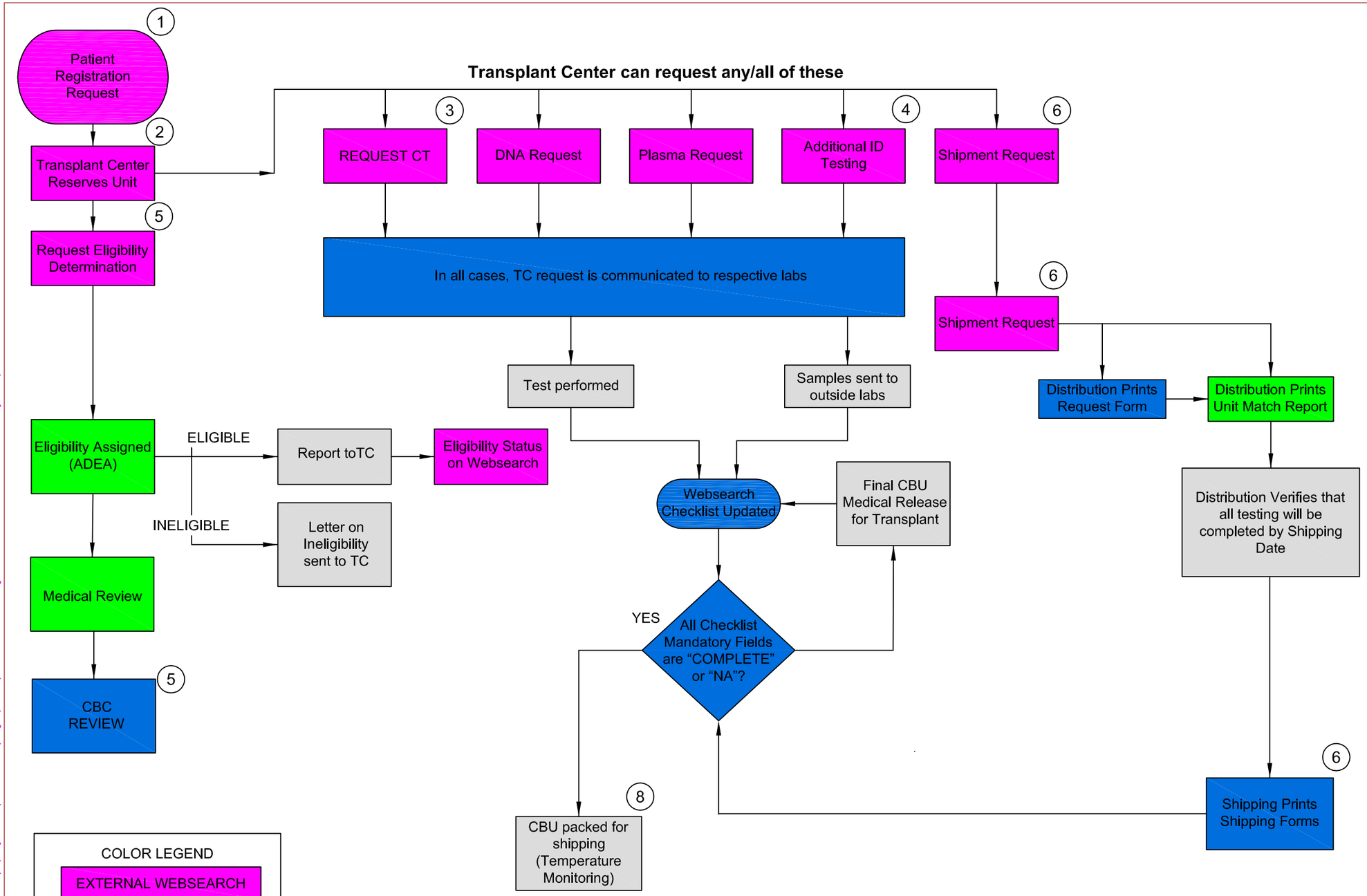

COLOR LEGEND

- EXTERNAL WEBSEARCH
- INTERNAL WEBSEARCH
- ORACLE FORMS
- MANUAL PROCESS

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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TITLE: WEBSEARCH

- PATIENT REGISTRATION
- ADDITION TESTING

DWG. NO: D1-1

REV. 1

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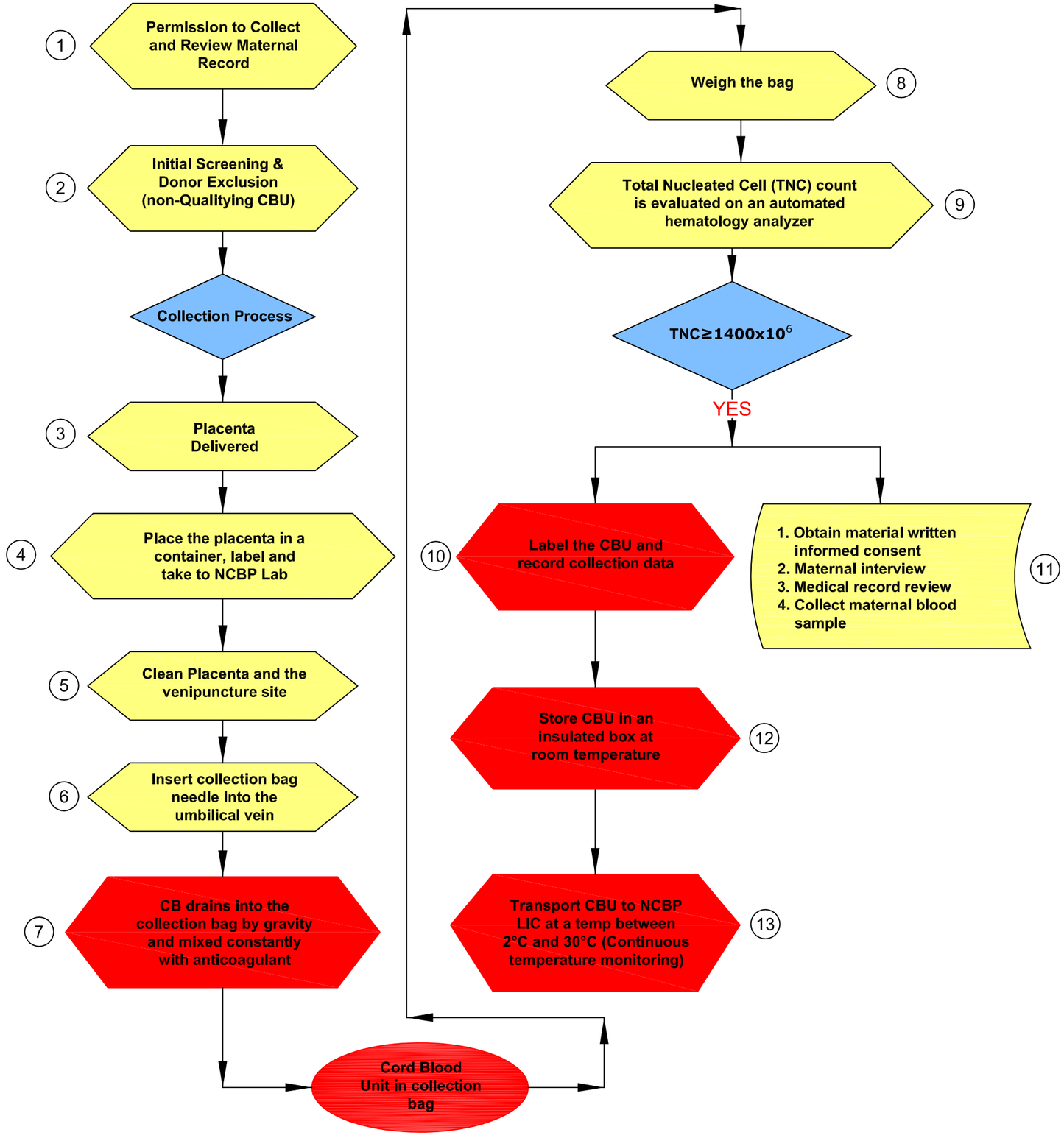

NATIONAL CORD BLOOD PROGRAM

New York Blood Center

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TITLE:

WORK FLOW DIAGRAM

COLLECTIONS

DWG. NO: W1-1

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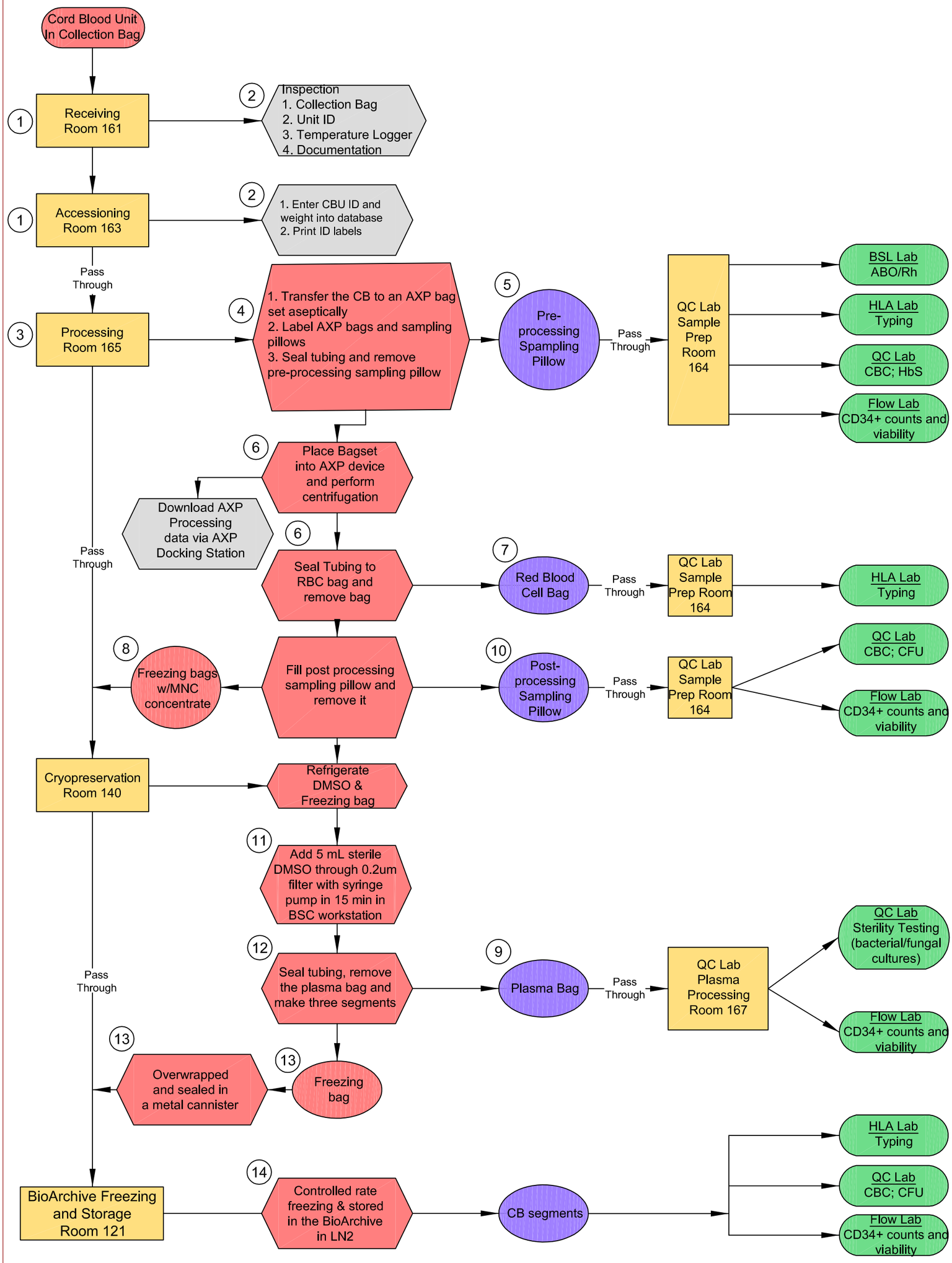

NATIONAL CORD BLOOD PROGRAM

New York Blood Center
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TITLE:

WORK FLOW DIAGRAM

PROCESSING

DWG. NO: W1-2

REV. 1

HPC-C DESCRIPTION AND CHARACTERIZATION

Hematopoietic Progenitor Cells, Cord (HPC-C, .the licensed CB products) manufactured and issued by the National Cord Blood Program of the New York Blood Center are minimally manipulated cellular biologic products that contain live human cord blood cells after volume reduction and partial Red Cell and Plasma depletion. The final cell suspension (20 mL) is cryopreserved by addition of 5 mL of 50% DMSO in 5% Dextran 40, so that the final concentration of DMSO is 10% and that of Dextran is 1%, is frozen at controlled rate, and stored in liquid nitrogen (-196°C) to preserve the cell viability.

NCBP HPC-C products are intended for use for allogeneic hematopoietic stem cell transplantation in the treatment of patients with the diseases specified in the FDA Guidance, namely, hematologic malignancies, certain lysosomal storage and peroxisomal enzyme deficiency disorders [(Hurler Syndome (MPS I), Krabbe Disease (Globoid Leukodystrophy), X-linked Adrenoleukodystrophy], primary immunodeficiency diseases, bone marrow failure and beta thalassemia, as recommended by the FDA Guidance for Licensure (Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic Reconstitution for Specified Indications).

The hematopoietic stem and progenitor cells contained in an HPC-C product, after infusion to an appropriately prepared ("conditioned") recipient, regenerate and replace the recipient's blood and immune system. Overall, allogeneic hematopoietic stem cell transplantation, a) allows treatment of malignancies, after high doses of radiation and chemotherapy that are capable of destroying also the recipient's bone marrow, through reconstitution with new, healthy hematopoietic cells. The reconstituted system can also exert an immunologic effect on the remaining malignant cells and prevent relapse; or b) in cases of genetic diseases, transplantation replaces the patient's defective cells with healthy ones that provide normal proteins (e.g. hemoglobin or immunoglobulins) or normally functioning enzymes that can restore the recipient's defective systems.

The label of each individual HPC-C unit (after License is granted) provides information about the total nucleated cell content, post-processing viability and viable CD34+ cells contained. The minimum nucleated cell content is 5.0×10^8 total nucleated cells, with post-processing viability of at least 85%, and the minimum CD34+ cell content is 1.25×10^6 viable CD34+ cells. HPC-C products have been screened for and are free of bacterial/fungal contamination. Donors have been screened for and are free of communicable Infectious Diseases.

HPC-C units are cryopreserved in two-compartment freezing bags. The larger compartment contains 80% of the cell suspension (20 mL) and the smaller contains 20% (5 mL). The HPC-C bag is placed and maintained inside a protective metal canister. Each HPC-C unit may be cryopreserved and frozen in 1 or 2 two-compartment bags, depending on the number of the total cells and the hematocrit at collection. This information is shown on the HPC-C label.

All cryopreserved HPC-C products, enclosed in labeled stainless steel canisters and protected by plastic foam thermal sleeves, are shipped frozen in special shipping containers (Dry-

Shippers) designed to maintain a controlled environment and a very low temperature (T ≤ -150ºC), electronically monitored and recorded for the entire transit time. Attachments to each shipment provide complete identification of the product, its contents and intended recipient and detailed instructions regarding the thawing of the product, dilution and re-suspension of the cells and infusion to the intended recipient.

OUTCOME AND SAFETY DATA FROM THE TRANSPLANTATION OF CB UNITS MANUFACTURED BY THE NATIONAL CORD BLOOD PROGRAM (NCBP)

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INTRODUCTION

In this section, we will present outcome and safety data from all NCBP units transplanted during the period 1993-2011, that have been processed with the 4 Processing Methods. We discuss particularly the AXP-processed units (Processing Method 4; the current one for which we are applying for a license) in the safety sections. We are presenting the outcomes of patients transplanted for diseases that are included in the indications, as recommended in the FDA Guidance for Licensure. (Of note, 4% of the recipients were transplanted for other indications). In the final section, we also present our view of the use of CB grafts for reconstitution of the hematopoietic and immune system, and/or correction of genetic structural or functional deficits of specific cellular enzymes and hemoglobin.

1.0 COLLECTION AND ORGANIZATION OF OUTCOMES DATA

1.1 Transplant outcome data for CB units manufactured by the NCBP

The purposes of obtaining information on transplantation outcomes are:

a) to monitor the clinical performance of the CB units,
b) to assess factors that affect the overall transplant outcomes, and
c) to evaluate the safety of the CB units during and after infusion to recipients, and monitor, investigate and document potentially serious adverse events.

1.2 Collection and evaluation of post-transplant information

NCBP Period: 1993 - 2007

Transplant and post-transplant questionnaires were sent directly to the transplant centers at certain intervals after transplant and information was completed by data managers or physicians. Data were evaluated at NCBP for completeness and accuracy; discrepancies or ambiguities were resolved with the transplant centers. Information and clarifications were also obtained by fax or email. All procedures are described in detail in the respective SOP for the collection of Post-transplant Outcome Information. Following these procedures, NCBP had outcomes information on over 90% of the units transplanted in this period.

Information on serious, life-threatening infusion reactions was requested on the Transplant Report, (CB Infusion in page 3). If a positive response was noted, additional information was obtained (which included copies of hospital records and laboratory test reports as needed) and evaluated according to the NCBP Form “Life threatening Post-Infusion Reactions”. A thorough investigation of the CB unit’s records (collection,

processing, testing, cryopreservation and shipping) was also performed and results were documented.

NCBP Period: 2008 - currently

The Stem Cell Therapeutic and Research Act of 2005 established the CW Bill Young Transplantation Program. Under this Program, the Stem Cell Therapeutic Outcomes database (SCTOD) administered by the Center for International Blood and Marrow Transplant Research (CIBMTR), was charged with collecting, analyzing and reporting on outcomes of all allogeneic transplants in the US, including CB transplants. CIBMTR must also provide accrued data to the CB Banks in electronic format monthly. Details on the data collection systems, forms, timeline, and auditing procedures are provided in the attachment "Overview of CIBMTR Cord Bloods Data Collection".

While CIBMTR started CB outcomes data reporting from all transplant centers in January 2008, there were significant delays in the collection, organization and verification of data. The procedures have improved, but still outcome information is not available for a proportion of the CB units transplanted in the US/Canada.

The data provided by the CIBMTR are evaluated at NCBP for accuracy in CBU identification, patient identification, infusion date, infusion of single or double unit graft. No "raw data" or hard copies are provided with the exception of thawing data on some CB units which, when submitted by the transplant centers, are also provided to the NCBP by email. The information is then transferred to the NCBP Outcomes database.

The CIBMTR did not have a mechanism for timely reporting of adverse events (now under development), these are reported in the CIBMTR forms as "adverse event summaries" (as described in the CIBMTR overview) but at no specific time intervals, and with no additional information, so that the severity cannot always be accurately evaluated by the NCBP.

Transplant centers are requested to report adverse events to the Cord Blood Coordinating Center (CBCC), according to the Stem Cell Act, or directly to the CB bank.

At the current time, NCBP receives notification and information about adverse events from the CBCC or the transplant center directly by email to the Medical Director. NCBP responds to the information within 24 hours.

The NCBP procedure for the evaluation is performed according to the "Life-threatening Post-Infusion Reactions" form. NCBP also provides additional documentation, as requested, to the CBCC and the transplant center, and may request data and reports from the Transplant Center to complete the evaluation and documentation of the events.

1.3 NCBP Outcomes Database

All data organization and evaluation is performed by the Medical-Transplant Follow-up group under the supervision of the Medical Director. The post-transplant information is organized in the NCBP Outcomes Database, which includes data obtained from CIBMTR, directly from the transplant centers, laboratory information, etc. Reference materials, relevant publications used for the organization of the database (e.g. guidelines for the determination of cause of death, medication doses qualifying for "non-myeloablative"

regimens, and other definitions), as well as details on event assignments, etc, are maintained in a Reference Manual that is updated annually.

Analyses are performed by the Medical-Transplant Follow-up group; expert statistical assistance is obtained from collaborative efforts or from consultants as needed.

Summary data on age distribution of the NCBP CB recipients (Table 1), matching between patient and donor CB unit (Table 2), and diagnoses of the transplanted patients (Table 3) are shown below for a total of 3532 patients and 3946 CB units (as of February 2011).

Table 1: Age distribution of the transplant recipients in the NCBP Database

Age groups (yrs)	N	% (of total)
<10*	1689	47.8%
10 - 20	642	18.2%
20 - 30	300	8.5%
30 - 40	276	7.9%
40 - 50	270	7.6%
>50	338	9.6%
(unknown)	17	0.5%
Total	**3532**	

For this analysis, patients with two transplants are counted at first transplant only)

Table 2: HLA match level between transplanted CBU and recipients

HLA Matches	(CB units)
2*	10
3*	114
4	2032
5	1529
6	261
Total	3946

For the matching, HLA-A and -B are considered at the low/intermediate, resolution level; DRB1 is considered at the allele level

Note: In the early years of CB transplantation 3/6 matched grafts were used occasionally; the 2/6 matches were finalized after the transplant was performed.

Table 3: Diagnosis of the recipients of the NCBP CB units

Diagnosis Group	N		% (of total)	(% of disease group)
Leukemia	**2177**		**61.6%**	
ANLL		**960**		**44.1%**
ALL		**916**		**42.1%**
Chronic leukemias		**301**		**13.8%**
MDS	**192**		**5.4%**	
Lymphoma	**228**		**6.5%**	
BM failure	**246**		**7.0%**	
Histiocytosis	**87**		**2.5%**	
Metabolic/storage disease	**223**		**6.3%**	
Krabbe		**22**		**9.9%**
ALD		**38**		**17.0%**
Hurler's		**51**		**22.9%**
immunodeficiency	**212**		**6.0%**	
Hemoglobinopathy	**56**		**1.6%**	
SCD		**23**		**41.1%**
thalassemia		**33**		**58.9%**
platelet disorders	**5**		**0.1%**	
neutrophil disorders	**32**		**0.9%**	
other malignancy (multiple myeloma, neuroblastoma	**29**		**0.8%**	
other	**3**		**0.1%**	
(unknown)	32		0.9%	
Total	***3532***			

Diagnosis Group		
malignant	**2621**	**74.2%**
non-malignant	**869**	**24.6%**
(unknown)	42	1.2%
Total	**3532**	

(for this analysis, patients with two transplants are counted at first transplant only)

1.4 Collaborations

It is the NCBP policy to perform collaborations with Transplant Centers and CIBMTR for analysis of data, meta-analyses and comparison of CB outcomes to those of bone marrow or peripheral stem cell grafts, and will continue to support such studies. Several of the publications discussed in this document are results of such collaborations.

1.5 ATTACHMENT: Overview of CIBMTR Cord Blood Data Collection

Comprehensive summary that describes the role of CIBMTR and the list of forms used for capturing the post-transplant information.

2.0 EVALUATION OF SAFETY OF THE NCBP CB UNITS

2.1 Adverse Events: CB unit Infusion Reactions

Since CB is a cryopreserved product thawed on the day on infusion, the majority of infusion reactions result from either red blood cells (RBCs) contained in the final product to be administered to the patient, or the cryoprotectant DMSO and/or the human albumin/dextran 40-containing diluents. The possible infusion reactions are described in detail in the Package Insert and may vary in severity from mild and easily treatable to severe and life-threatening:
Mild: reactions during or after the infusion of the CB product that require minor medical intervention and do not affect the overall patient status or outcome.
Severe: serious, life-threatening or fatal infusion reactions, requiring major medical intervention. These include anaphylactic shock, acute cardiac, pulmonary or renal failure, seizure, patient transfer to the Intensive care Unit, or death within 48 hours of the CB infusion. Additionally, un-expected adverse events would be categorized, followed and if necessary treated as serious.

Infusion reactions can be decreased or minimized by:

a. washing the CB unit after thawing to remove the bulk of lysed RBCs and the DMSO
b. alternatively diluting the thawed CB product, so that the concentration of red cell membranes, hemoglobin etc., and DMSO is lower, although the total amount remains the same
c. premedication and adequate hydration of the patient

NCBP provides instructions to the transplant centers about the thawing and preparation of the CB products for infusion. The instructions (included in the documents accompanying the unit to the transplant center) describe both methods: thaw with washing and removal of the DMSO, and thaw with albumin reconstitution, i.e., dilution without removal of the DMSO. NCBP does not recommend thaw and immediate infusion of the CB product without either washing or dilution.

2.1.1 Attachment: Procedure for cord blood thawing and preparation for administration (NCBP Instructions for transplant centers)

2.1.2 Frequency of post-infusion reactions

Overall infusion reactions are frequent.
Review of the summary data submitted to the NCBP by the CIBMTR shows a frequency of 19% (129 reactions for 670 units). The vast majority of those were mild. A recent clinical review of infusion reactions after double unit CB transplants from various Banks showed a higher frequency (41%: 45 reactions in a total of 108 CB units).[1] Similarly, most were mild, from known ("expected") causes and could be treated without complications.

2.1.3 Severity of post-infusion reactions

Serious Post-Infusion Reactions reported to the NCBP are summarized in the attachment. None of the events involved an-AXP-processed CBU.

2.1.4 Attachment: Summary of Serious Post-Infusion Reactions (SAE)

2.1.5 Infusion Reactions for the AXP-processed CB units

Note: AXP processing of the CB units results in a more "predictable" RBC depletion so that the post-processing hematocrit is <50% in all products.

Table 4: Infusion Reactions for the AXP-processed CB units

Severity of Infusion Reactions for AXP-processed CB units

Severity	Description of Adverse Infusion Reactions in AXP-processed CB products	N	% (of CBU transplanted)	% (of CBU with report)
0	no report, no post-thaw information (not evaluable)	**200**	**45%**	
1	no infusion reactions encountered	**204**	**46%**	**83%**
2	hypertension, nausea, vomiting	**28**	**6%**	**11%**
3	hypoxia, chest pain, shortness of breath, hemoglobinuria, hypotension	**13**	**3%**	**6%**
4	severe (hematuria, seizure,acute renal/cardiac/pulmonary failure or death within 48 h)	**0**		
	Total AXP -processed CB units transplanted	**445**		
	Total CB units with infusion data	**245**		

The majority of the reactions in the Table above have been included in the CIBMTR reports. As discussed above, CIBMTR does not have a mechanism for timely reporting of adverse events (this is now under development), and for submission of additional clinical and laboratory information so that the severity of the events can not be assessed accurately.
To address this, NCBP is proposing a system of direct inquiring of adverse events and infusion reactions post-licensing (Bank-initiated). Then, detailed data can be submitted to CIBMTR.

In summary, as described above, reactions after CB product infusion are relatively frequent and "expected" events. Severe life-threatening reactions have been rare.

2.2 Post-transplant Adverse Events

The post-transplant events are Engraftment Failure, Death and Relapse. The aim of the NCBP analyses has been to describe the frequency of these events, define the variables that affect the outcomes, and, through the participation in the CIBMTR studies, analyze them in comparison with other graft sources. These are described in details in sections 4.0 and 5.0.

In that context, and taking into consideration the current literature (over 30 recent publications), the following points can be made:

- Time to engraftment after CB transplants is slower than that after bone marrow (BM) or peripheral blood stem cell (PBSC) grafts. Furthermore, time to neutrophil and platelet recovery depends on the cell dose of the CB graft, with more rapid recovery in patient receiving products with high cell doses.
- Rates of engraftment failure have been reported as high as 20% in some studies of single unit grafts.
- To overcome the cell dose limitation of a single CB unit, many centers are employing double unit cord blood grafts with lower rates of primary graft failure.
- CB transplants do not require as stringent HLA match criteria as BM grafts. Transplants with CB grafts mismatched at 1 or 2 loci are being performed with acceptable levels of GvHD. As described above, certain mismatches (e.g., NIMA-matches, HLA homozygous MM) may offer improved outcomes.
- The overall incidence of acute GvHD in large series ranges from 33% to 44% for grade II-IV and from 11% to 22% for grades III-IV. The incidence of chronic GvHD ranges from 0 to 25%. This incidence, although not low, is particularly notable since most CB donor-recipient pairs have mismatches in 1-2 HLA loci.
- Importantly, there is no evidence to suggest a higher risk of relapse after CB transplantation. Relapse rates were not different in the comparison study of unrelated CB and unrelated BM transplants in pediatric or adult patients, as clearly was demonstrated in the CIBMTR studies.[2, 3]
- Donor (graft) derived leukemia (myelodysplastic syndrome / acute leukemia) are very rare complications of allogeneic transplantation; these have been described after BM and CB grafts. In a recent analysis the frequency after CB grafts was thought to be 0.4%. [4] Specific chromosomal translocations have been implicated, such as monosomy 7 and 11q23. Although a few cases on “new” chromosomal abnormalities post transplant have been reported to the NCBP, no clear diagnosis of leukemia has been established.

2.3 Potential transmission of infectious disease agents

Bacterial contamination: CB products available for transplantation have had negative bacterial and fungal cultures. There have been a few reports of positive bacterial cultures post-thaw; however, in all cases, the pre-cryopreservation cultures were negative. The contamination probably occurred with the handling of the unit at the transplant center

during thaw and preparation for infusion, or further manipulation (e.g. in the course of cell expansion).

Other infectious disease agents: CB donors (mothers and CB samples) are subjected to stringent screening procedures intended to reduce to minimum the risk for infectious agent transmission. Screening includes antibodies to hepatitis B, C, HIV 1 and 2, HTLV 1 and 2, Chagas disease, syphilis, surface antigen for hepatitis B and NAT for HIV/HCV and WNV. However, these measures cannot completely eliminate the risk of transmitting these agents.

Further, NCBP performs additional testing on CB samples and mothers, if needed. Examples include CMV PCR ('In house" assay) for all CB units when the mother is seropositive for CMV (total anti-CMV Ig positive), HBV PCR when the mother is positive for total anti-HB core antibody (and HbSAg negative), or Toxoplasmosis serology in the mother and Toxoplasmosis PCR in the CB sample if relevant history is obtained. There are no FDA-approved assays for these tests.

However, certain limitations exist: For example, there are no routine tests to predict or prevent disease transmission for some bloodborne infectious pathogens, such as Babesia species, Leismania species, Plasmodium species and viruses such as parvovirus, coronavirus (associated with SARs), agents for human transmissible spongiform encephalopathies (TSEs) and certain trypanosomes. Additionally, diseases caused by currently unknown agents may be detected in the future.

NCBP has not been notified for any transmission of infectious agents to the recipients.

2.4 Potential transmission of genetic diseases, including hemoglobinopathies

It is possible that certain genetic diseases may be transmitted though the cord blood to the patient. While these are very rare, cord blood can only be tested for a few of the many possible diseases. To reduce this possibility, cord blood is not collected from babies that have a family history of genetic diseases.

CB can be tested for hemoglobinopathies. Hemoglobinopathy screening of all HPC products is performed. Briefly, all CB products are screened for the presence of hemoglobins F, A, S, D, C and E by high performance liquid chromatography (HPLC) method using the VARIANT™ Sickle Cell Short Program manufactured by Bio-Rad Laboratories, Diagnostics Group. The Program is 510(k) approved for in-vitro diagnostic use.
All Sickle Cell "Trait" (heterozygous) or homozygous samples are retested to confirm the previous result. Hemoglobinopathy test results are reviewed prior to CB unit release to searchable inventory. All confirmed homozygous CBUs are permanently deferred.

Testing for alpha- and beta-thalassemia is performed at the Oakland Hospital Reference Laboratory for Hemoglobinopathies, by molecular testing. Samples are tested based on family history of hemoglobinopathy other than Sickle Cell Disease, complete blood counts (MCV value) and/or hemoglobin screening results.

We have not been notified about a case of transmission of a genetic disease to a recipient.

2.5 Other aspects of safety for the NCBP HPC products

2.5.1 Verification of CB unit Identity

Prior to release for transplantation, verification of the identity of the CB unit is performed by confirmatory HLA typing of a sample from the attached segment of the freezing bag, for antigen/allele assignments for HLA -A, -B and -C at low intermediate resolution and for -DRB1 at high resolution.

2.5.2 Verification of the donor and recipient identity

In cases where the patient and the donor CB unit have no HLA mismatches at the HLA-A, -B, -DRB1 loci, the following actions are undertaken to assure that the donor and recipient are not the same person:

- Review of dates of birth of patient and infant donor and their gender (blood group and Rh typing also, if they are available)
- Review of high resolution confirmatory typing of the HLA-A, -B and -C loci of patient and donor, since these may disclose differences not detected at low intermediate resolution
- Review of the patient's maternal HLA typing (if available, esp. in pediatric patients) and comparing it to the HLA typing of the mother of the CB donor can also be helpful.
- If no resolution is achieved, patient and donor have to be tested by a different method not based on HLA differences (e.g., Short Tandem Repeat, STR) to provide accurate genetic identification.

2.5.3 Stability of the NCBP HPC products

Since 1993, NCBP has used four processing methods for CB units. License is sought for the products manufactured with the last method, method 4 (AXP-processing). However, stability of units manufactured with older methods will continue to be evaluated, along with that of the current method. Evaluation is based on in vitro measurements of integrity, identity, sterility and potency with validated methods. Additionally, evaluation includes review of clinical data from the transplanted CBUs.

The following Table shows the number of transplanted CBU from each processing period and their storage time. The oldest NCBP unit that has been transplanted with proof of successful engraftment was cryopreserved for a total of 13 years. Further, the study below summarizes comparison of outcomes of units transplanted after long storage time (> 8 years) to those of recently collected units (storage time <2 years).

Table 5: Storage time of the transplanted CB units

Manufacturing Method	1	2	3	4
Time period	**1993 - 1995**	**1995 - 05/1999**	**05/1999 - 08/2006**	**08/2006 - 08/2011**
Number of transplanted CBU	465	902	2240	557
Number of Transplants	463	887	1981	481
Number of Patients	448	866	1935	467
Storage Time* (N, %)				
< 1 year	18 (4%)	139 (15%)	297 (13%)	145 (26%)
1 - 5 years	332 (71%)	536 (59%)	1447 (65%)	412 (74%)
5 - 10 years	94 (20%)	167 (19%)	486 (22%)	0 (0%)
10 - 15 years	20 (4%)	59 (7%)	10 (0%)	0 (0%)
> 15 years	1 (0%)	1 (0%)	0 (0%)	0 (0%)
median time in storage (years)	*3.64*	*2.80*	*2.88*	*1.59*

Number of CBU is higher than the number of transplants reflecting transplants with double unit grafts.
Number of patients is smaller than the number of transplants, because some patients received two transplants.
*Storage time: time from processing to infusion, indicating the period that the CBU has been cryopreserved ("age" of the CB unit).

Comparison of transplant outcomes of CB units cryopreserved for longer than 8 years to those of recently stored units (< 2 years) (Figures 1, 2)

The NCBP conducted a study to evaluate the quality of CB units after prolonged storage in liquid nitrogen freezers. For that, the transplant outcomes of patients that received CB grafts from the New York Blood Center NCBP stored for 8 years or more (Group 1, N=43 patients, median time from unit collection to transplant 9.2 years) to those transplanted with units stored < 2 years (Group 2, N=300 patients, median time in storage: 1.1 years) were compared.[5]
Patients were included in the study if they were transplanted in the period 2001-2006, received a single or double unexpanded unit graft and had no prior transplant in the 6 months preceding the CB graft. Double unit recipients were evaluated if the unit of interest was the one that engrafted, as demonstrated by post-transplant chimerism studies.

No differences were identified in the time to engraftment (Figure 1); Transplant-related mortality (Figure 2) or rates of engraftment failure (Table 6).[5]
These results indicate that CB units processed and cryopreserved using processing methods 1 and 2 can be used effectively in clinical transplantation. Therefore, long-term cryopreservation of CB stem cells is feasible without compromising the quality and engraftment ability of the CB units.

Figure 1: ANC Recovery by Storage Time of the Graft

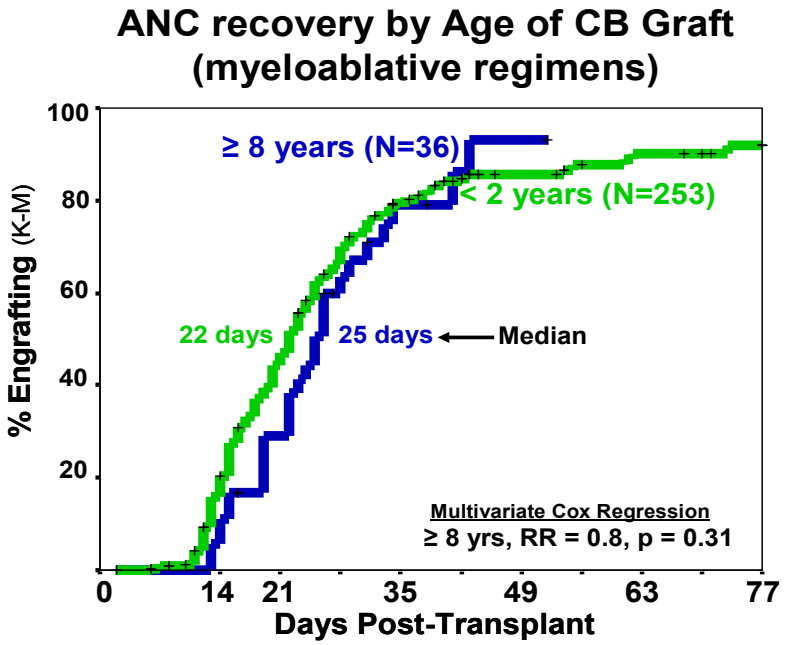


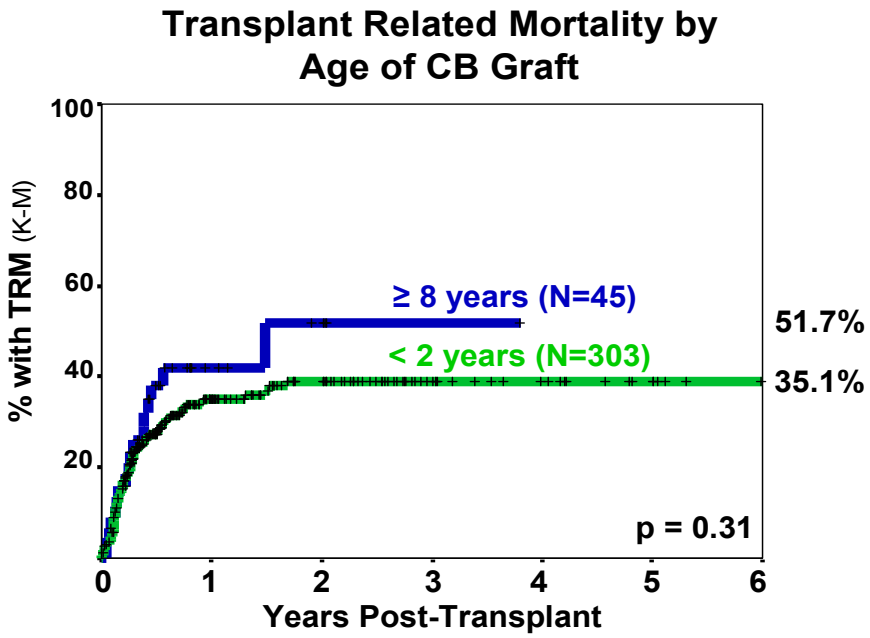
Table 6: Effect of storage time of the CB unit on engraftment

	All	ANC>500	Graft failure	Early death
Study (N)	**45**	**33**	**8**	**4**
Control (N)	**303**	**244**	**34**	**19**
				p:0.35

Graft Failure: No donor-derived ANC by day+42
Early death: death before day+42 without engraftment
ANC>500: 1 pt (study) and 6 pts (control) after day+42 (6 unknown from control group)

No differences were identified in the time to engraftment (Figure 1); Transplant-related mortality (Figure 2) or rates of engraftment failure (Table 6).[5] These results indicate that CB units processed and cryopreserved using processing methods 1 and 2 can be used effectively in clinical transplantation. Therefore, long-term cryopreservation of CB stem cells is feasible without compromising the quality and engraftment ability of the CB units.

Figure 2: Transplant Related Mortality by Storage Time of the CB Graft



3.0 ANALYSIS OF NCBP CB OUTCOMES DATA

3.1 NCBP outcomes data submitted to the FDA Public Docket

The first comprehensive transplant outcome report of the NCBP was published in the NEJM in 1998, describing the outcomes of the first 562 patients who were transplanted with CB units in the US and abroad.[6] Subsequently, the detailed data set was submitted to the FDA public docket (1997N-0497).

3.2 IND Reports to the FDA

NCBP (then named Placental Cord Blood Program) was granted an IND from the FDA in 1996 "Placental Blood for Marrow Replacement". The annual Reports summarize the outcome data with specific emphasis on events: Death, Relapse and Graft failure. In those reports, un-expected or severe adverse events related to CB infusions are also being reported.

3.3 NCBP analyses of outcomes data, and selected publications

For the analysis of post-transplant information, the following outcome endpoints are considered, according to standard definitions (also details are provided in the NCBP Outcome Analysis Plan):

Engraftment: Time to myeloid engraftment is defined as the first of three consecutive days of absolute neutrophil cell count ≥500/µl (ANC>500).
Note: for the evaluation of engraftment the cytoreduction regimen needs to be taken under consideration. The myeloablative cytoreduction regimen "empties" the patient's bone marrow, so all peripheral blood cells after the transplant are derived from the graft. In cases on non-myeloablative conditioning, some of the peripheral blood cells initially derive from the patients' own residual marrow and donor cell engraftment must be verified by chimerism studies.
Primary graft failure was defined as either:

a) never having achieved ANC>500 within the time interval defined according to the transplant center procedures, or
b) ANC>500 with no donor engraftment by chimerism studies (**autologous recovery**).

Survival: Overall survival and the exact opposite variable, **Transplant-Related Mortality** (TRM) have been evaluated in all studies. For patients with hematologic malignancies, TRM is defined as death from any cause, while the patient was in remission. Among patients without hematological malignancies all deaths are considered transplant-related. Surviving patients are censored at the time of last follow up.

Relapse: in patients with hematologic malignancies, diagnosis of relapse is based on clinical or cytogenetic relapse. In patients with non-hematologic malignancies, disease "recurrence" is defined in cases of autologous recovery.

Graft-versus- Host Disease: Acute Graft-versus-Host Disease is diagnosed by the transplant centers; grades of acute GvHD (I-IV) and chronic (limited or extensive) are used as assigned by the transplant centers.

In the outcome analyses, autologous recovery, relapse and death are considered **events**.

The probabilities of engraftment, acute GvHD, transplant related mortality and relapse rates are estimated in most studies using cumulative incidence methods and considering competing events. For example, in evaluating the incidence of post-transplant relapse, death is a competing event.
Multivariate models (Cox regression analyses) have been used in most studies to evaluate the relative risks, 95% confidence intervals and statistical significance of various differences.

Attachment: NCBP Outcomes Analysis Plan

NCBP publications

After the original NEJM study,[6] several other studies analyzed transplant outcomes in large patient cohorts, mainly evaluating the effect on transplant outcome of two of the most important graft characteristics:

a) TNC dose (Total Nucleated Cell content of the CB product pre-cryopreservation divided by the patient's weight in kg) and
b) HLA mismatch level (MM); HLA match was evaluated for HLA-A and -B antigens at the intermediate level of resolution, match for -DRB1 was defined at high resolution allele level.
c) Additional studies have evaluated the different HLA mismatch types (direction of HLA mismatch, or HLA mismatch that matches the donor's non-inherited maternal antigen -NIMA), and their effect on transplantation outcomes.

Selected analyses from these studies are presented in the following sections.

4.0 NCBP OBSERVATIONAL STUDIES

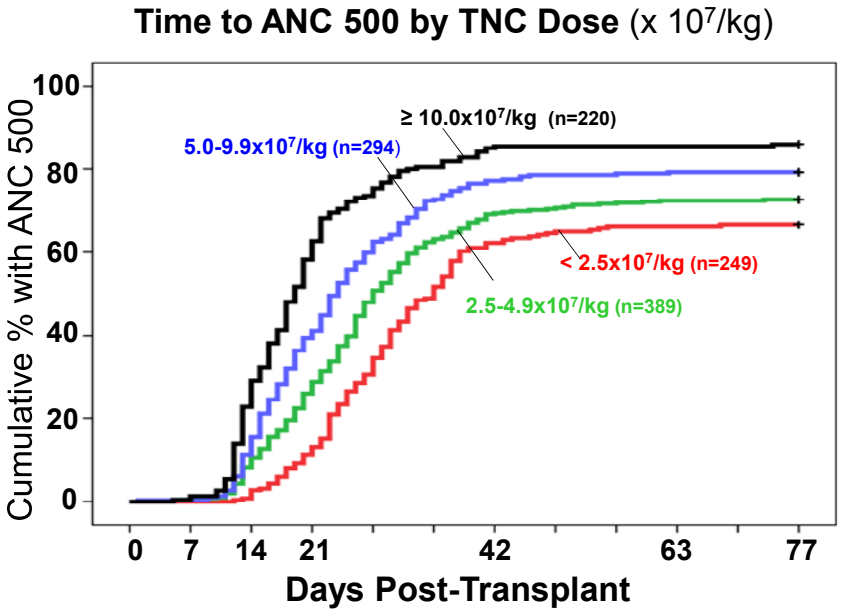
Selected analyses are summarized here with emphasis on engraftment and transplant related mortality and the respective references are provided. These results are from published studies; details on study inclusion criteria and statistical analyses are provided in the Methods sections of the referenced manuscripts.

4.1 Effect of CB unit cell dose (TNC/kg) on engraftment

Engraftment (ANC>500) in patients transplanted with single unit CB grafts: time and probability of engraftment with different TNC doses (Figure 3)

Figure 3 shows the cumulative incidence of engraftment in recipients of single unit CB grafts (N=1202, all diagnoses) stratified according to the TNC/kg. A small proportion of patients (N=220, 17%, mainly very young children) received high TNC doses (>10x10^7/kg) and they seem to have the best outcomes.[7] A step-wise decrease in the probability of engraftment is seen with decreasing TNC doses.

Figure 3: Time to Engraftment by TNC dose of the CB unit
(ref.[7])



The improved speed of engraftment (defined as time to ANC>500; first of three consecutive days) as well as improved probability of engraftment with increasing TNC doses has been seen in all NCBP analyses, as well as other analyses from other Registries and transplant centers.

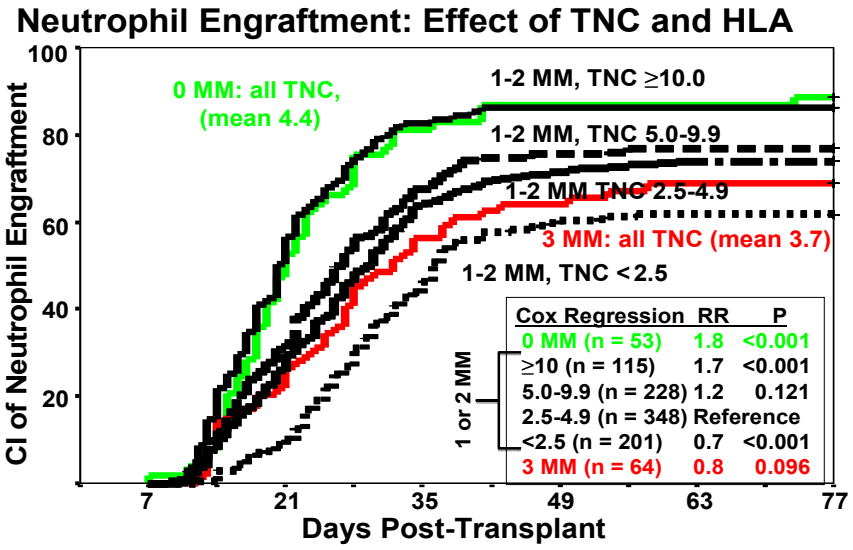
As a result, the cell dose of the CB is a very important graft characteristic for the selection of CB units for transplantation.

The other critical graft characteristic is the HLA match (or number of mismatched antigens between donor CB unit and recipient.

Interaction of TNC and HLA match in engraftment for patients with hematologic malignancies who received single unit grafts (Figure 4)

The study evaluated the combined effect of TNC and HLA match on transplant outcomes.[8] As seen in Figure 4, the best results were obtained with 0 mismatched grafts (with TNC doses 0.7-19.4x10^7/kg, mean 4.4x10^7/kg), or 1 or 2 HLA MM grafts with high TNC dose >10x10^7/kg. A decrease in the probability of engraftment and a longer time to achieve ANC>500 ("shift of the curve to the right") was seen with 1-2 MM units with lower TNC dose.

Figure 4: Combined effect of TNC and HLA on Engraftment of CB units
(ref. [8])

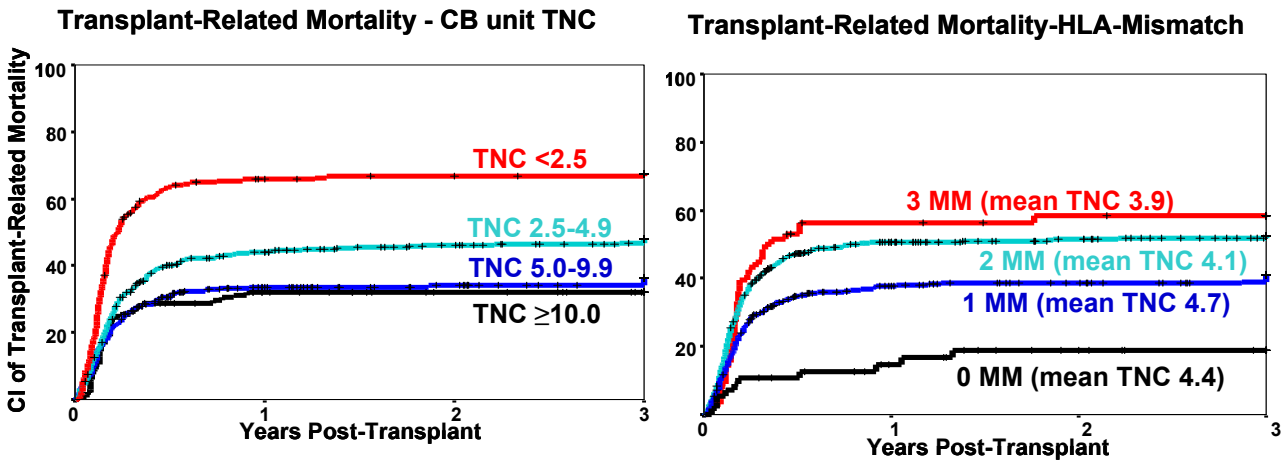


4.2 Effect of TNC and HLA match on Transplant Related Mortality (TRM)

Transplant - Related Mortality, i.e., death from all causes other than relapse, and effect of TNC or HLA match level of the graft (Figures 5 and 6) ref[8]

Figure 5: Effect of TNC dose of the CB graft on Transplant Related Mortality

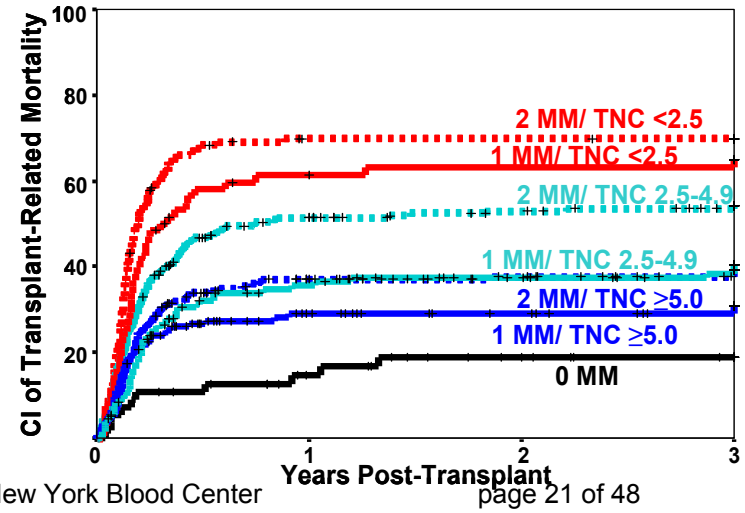
Figure 6: Effect of the donor-recipient HLA match on transplant-related mortality



Cumulative incidence of TRM for a total of 1061 patients with hematologic malignancies, who received single unit grafts after myeloablative cytoreduction is shown. TRM was evaluated based on CB unit's TNC dose (Figure 5) and HLA match level (Figure 6). CB units with TNC<2.5x10^7/kg have the highest TRM. In regards to HLA mismatch level, early transplants had used 3/6 matched units (6% of total); this is not the current practice given the high risk of serious complications and mortality with this level of MM, and the availability of better matched units for most of patients.

Figure 7: Combined effect of TNC and HLA of the CB graft on TRM

TRM: TNC and HLA-Mismatch Combined



Relative Risk and p values

TNC & Match	N	RR (95%CI)	p
0 MM (all doses)	56	0.5 (0.2-0.9)	*0.022*
1 MM ≥5.0	155	0.8 (0.6-1.3)	0.413
2 MM ≥5.0	212	1.1 (0.7-1.5)	0.782
1 MM 2.5-4.9	132	Reference	
2 MM 2.4-4.9	240	1.5 (1.1-2.1)	*0.011*
1 MM <2.5	68	1.9 (1.3-2.9)	*0.002*
2 MM <2.5	139	2.4 (1.7-3.4)	*<0.001*
3 MM (all doses)	66	1.7 (1.1-2.6)	*0.017*

To evaluate the effect of combined TNC and HLA match on TRM,[8] the patients that received 1 MM grafts with TNC dose 2.5-4.9x10^7/kg were used as the reference group. TRM was not different in recipients of CB units with TNC dose >5x10^7/kg and either 1 or 2 MM, compared to the reference. However, patients given units with 2 MM and TNC 2.5-4.9x10^7/kg had higher TRM than those receiving the same cell dose with 1 MM. Furthermore, no significant difference the TRM was seen among patients who received units with 1 MM and TNC 2.5-4.9x10^7/kg and those with 2 MM and TNC>5x10^7/kg. Thus, lower TRM was achieved with better HLA matches without an increase in relapse rates. In other words, no advantage in selecting 4/6 matched units (2 MM) in order to increase the anti-leukemic effect of the graft was seen in this study.
The superior outcome of the 6/6 M grafts was confirmed by this analysis.

4.3 Effect of donor-recipient HLA match on transplant outcomes

The effect of HLA match, or alternatively the number of MM (mismatches) on the transplant outcomes has been evaluated in several analyses. Results have shown that 0 MM CB grafts (i.e., matched at all 6 HLA loci) have the best outcomes. However, only 10-15% of the patients will have a fully matched (0 MM) CB unit, even with the increased global Inventory (World Marrow Donor Association - WMDA data 2010). As a result, it becomes very important to evaluate whether certain MM can have better outcomes that others. NCBP has performed two large analyses evaluating the MM between the donor CB unit and the recipient, and their outcomes.

Selection of HLA-mismatched grafts: Improved outcomes with HLA-mismatched but NIMA-matched grafts (NIMA: non-inherited maternal antigens) in patients with hematologic malignancies (Figures 8, 9)

In certain patient-unit pairs, the patient's mismatched antigen may be the same as the donor-mother's antigen at the same locus (example below). These "*NIMA matched*" combinations may happen by chance in cases of common HLA antigens; retrospective evaluation of 1121 patient-unit pairs revealed that 79 (7%) had received NIMA matched grafts. [9] This analysis was possible because NCBP performs HLA typing of the maternal sample for all CB units to be released for transplantation.

An example of an HLA MM, NIMA matched CB unit-recipient pair is shown below

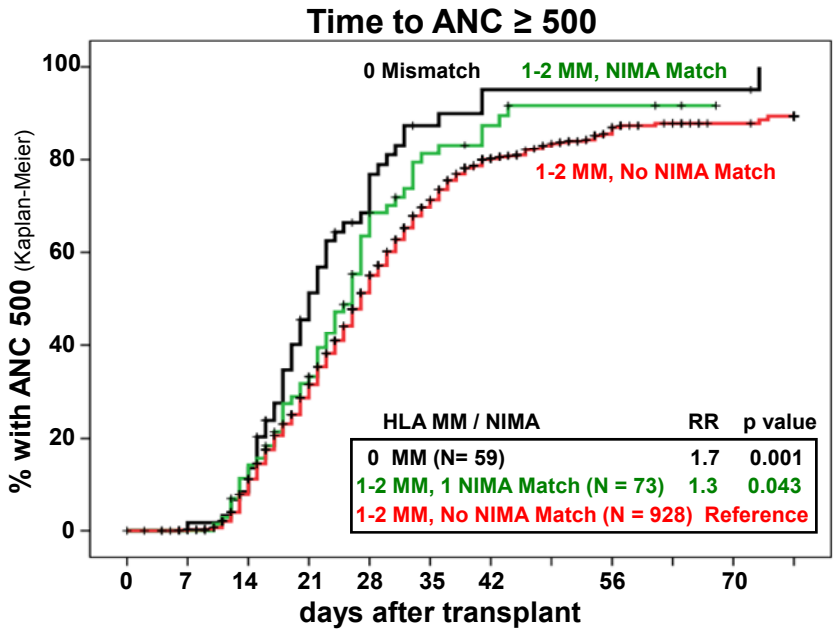
HLA mismatch, NIMA match CB unit

Mother of CB	**A1, A2**	**B7, B8**	**NIMA: A2, B8**
CB unit	**A1, A3**	**B7, B44**	
Patient	**A1, A2**	**B7, B44**	**HLA-MM, NIMA M: A2**

M: matched
MM: mismatched
NIMA: Non-Inherited Maternal Antigen

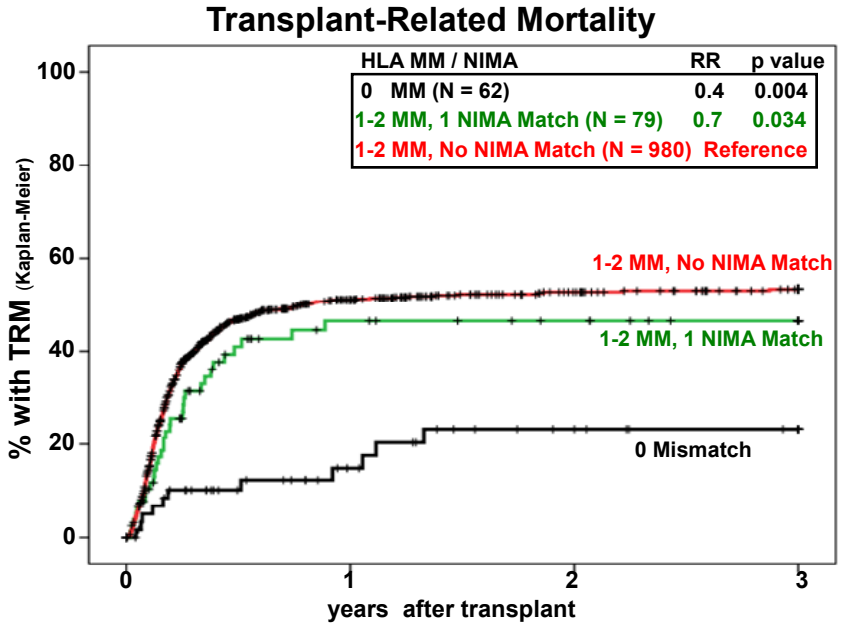
Patient and CB unit differ at one A locus: patient has A2 while the CB unit has A3. The patient's A2 antigen is the same as the CB donor mother's antigen at the same locus (NIMA match. [9])

Figure 8: Time to Engraftment for NIMA-matched and NIMA-mismatched CB grafts



Patients who received NIMA matched transplants were shown to have faster engraftment (Figure 8) and lower transplant-related mortality (as shown in Figure 9), overall mortality and treatment failure (the opposite of disease-free survival). Their outcomes were similar to those who received fully matched grafts ("0 MM").[9]

Figure 9: TRM for NIMA-matched and NIMA-mismatched CB grafts



The ability to use mismatched CB grafts with outcome similar to those of fully matched grafts increases the probability of finding optimal grafts for patients, particularly those with uncommon HLA antigens.[9]

Selection of HLA-mismatched grafts: Effect of direction of the HLA mismatch and HLA homozygosity on outcomes, in patients with hematologic malignancies and other diseases (Figures 10, 11)

The effect of the direction of HLA MM was evaluated,[7] with the hypothesis that vector effects should be most apparent in unidirectional mismatches. By definition, when a mismatched HLA antigen is present in both recipient and donor, the mismatch is bidirectional. In contrast, if either the donor or the recipient is homozygous for the same HLA antigen in one locus, the MM is unidirectional. Thus, if the *donor* is homozygous at a locus but the patient is not, only donor cells have an HLA target (GVHD direction) at that locus. Conversely, if the *patient* is homozygous for a shared antigen at the mismatched locus but the donor is not, only host cells have an HLA target on the graft (HVG or rejection direction).

Examples are shown below:

Unidirectional HLA mismatches

	5/6 Match: GvHD Direction (no rejection mismatch)			5/6 Match: Rejection Direction (no GvHD mismatch)		
	A	B	DRB1*	A	B	DRB1*
Pt	2, 3	7, 44	0401, 1501	2, _	7, 58	1102, 1503
CB	2, _	7, 44	0401, 1501	2, 30	7, 58	1102, 1503

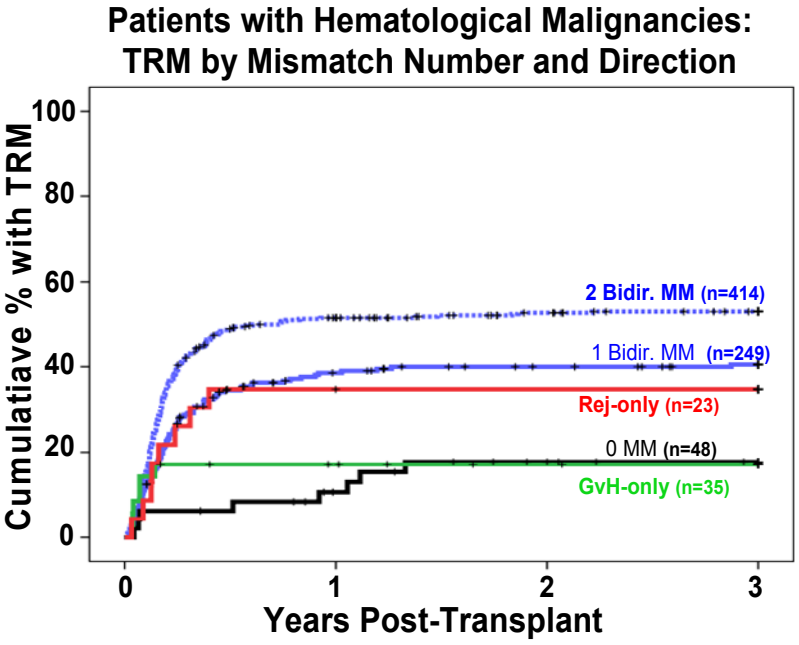
CB unit has a "Blank" at A locus (Homozygous for A2)

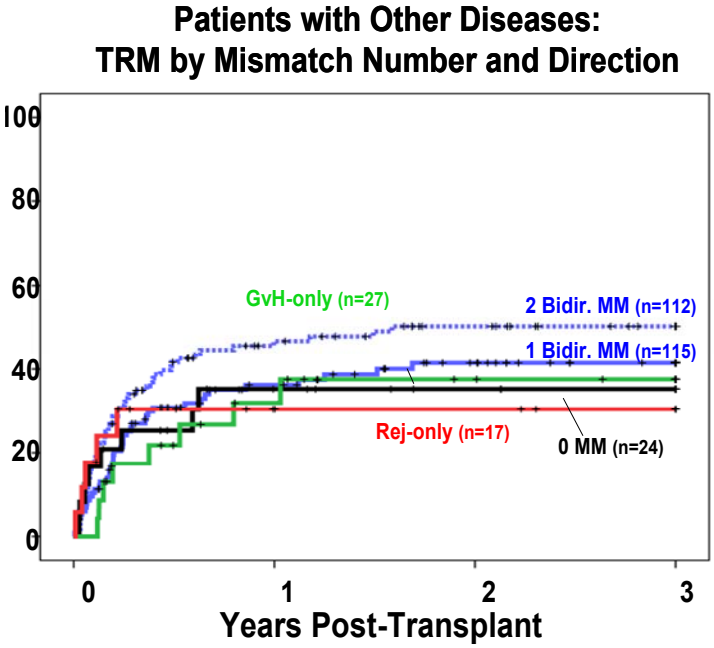
Patient has a "Blank" at A locus (Homozygous for A2)

Evaluation of 1202 patients transplanted with single CB units during the period 1993-2006 revealed 98 patient-donor pairs (8.1%) that had unidirectional MM: 58 in the GvHD direction only (GvH-Only MM) and 40 in the rejection direction only (Rejection-Only MM). Additionally, seventy patients had 0 MM grafts and the remaining had bidirectional (or combination of bi- and unidirectional) MM. Analyses showed associations between mismatch direction and transplant outcomes, when all mismatches were unidirectional. GVH-Only MM grafts had outcomes that were as good as those that were fully matched for HLA-A, -B and -DRB1 antigens. Rejection-Only MM grafts, on the other hand, carried a higher risk of relapse and lower engraftment rate. [7]

Figure 10: TRM by HLA MM direction in hematologic malignancies

Figure 11: TRM by HLA MM direction in patients with other diseases





4.4 NCBP-CIBMTR studies.

Differences were identified in the outcomes of CB recipients compared to those transplanted with BM/PB in the time to engraft and transplant-related mortality when the CB grafts had low cell doses.[2,3,10] Furthermore, a very important conclusion of these studies was that the relapse rates were not different among the different grafts, so that CB recipients had similar leukemia-free outcomes to those of BM.

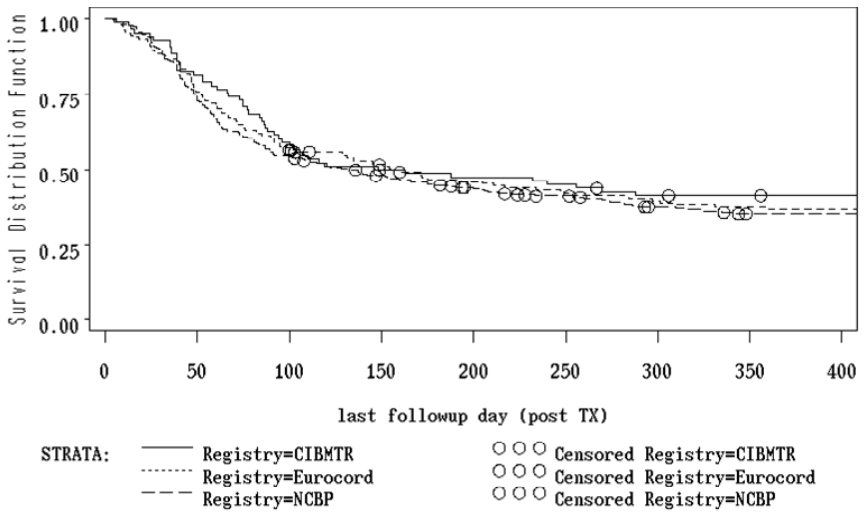
Besides the clinical usefulness of the analyses, these studies allowed the comparison of the NCBP collected data directly from the transplant centers (prior to 2008) to those reported by the transplant centers to CIBMTR during the same period. Very few discrepancies were identified (in <5% of patients), almost all of which were subsequently resolved by consultation with the transplant centers confirming the discrepant versions of each in about half the contested cases.

4.5 Combined outcome data from 3 Registries (CIBMTR, Eurocord and NCBP).

The first study[11] evaluated the factors affecting mortality in a total of 514 adult patients with hematologic malignancies who received cord blood transplants after myeloablative conditioning. NCBP contributed outcome data for 262 patients (51% of total). Despite differences in the clinical practice of transplantation, the survival results were remarkably similar, as shown in Figure 12.

Figure 12: Post-transplant Mortality with CB grafts: data from 3 Registries

Kaplan-Meier survival curve (to 365 days) by registry



In the second study, [12] the combined data of the 3 Registries were used to evaluate the CB transplant outcomes in patients with hemoglobinopathies (35 with thalassemia and 16

with sickle cell disease). NCBP contributed outcome data for 19 patients (37% of total) in the study. Primary graft failure was the predominant cause of treatment failure. As described in the study, primary treatment failure can be attributed to a variety of recipient-related as well as graft-related causes but this particular patient population is at high risk because of the multiple prior transfusions and possible allosensitization. Relative high graft failure rates have been observed with BM transplants in similar patient cohorts.

4.6 Evaluation of outcomes in disease-specific patient groups

Outcome analyses in patients with Hurler's syndrome,[13] and other metabolic diseases,[14,15,16] after transplantation with unrelated CB grafts (many provided by the NCBP) have been reported by transplant centers, with very good results, in regards to correction of the metabolic abnormalities as well as prevention or improvement of the neuro-cognitive sequelae of the underlying disease. In these cohorts, very young patients (including neonates) were included. The authors noted that patients who underwent transplantation as newborns had better functional outcomes than those with early infantile forms of the disease with progressive symptoms.[14] (More information is provided in section 5.0)

4.7 Double unit CB grafts

To overcome the cell dose limitation of the single CB units, several centers have employed transplantation with double unit CB grafts, with encouraging results on engraftment, transplant-related mortality and leukemia-free survival. A large national randomized study is in progress to evaluate the advantages and disadvantages of single versus double CB grafts in pediatric patients. In two recent studies engraftment was reported to be over 90% in recipients of double unit CB grafts.[17,18] Of note that, in the MSKCC study, 63 (75%) of the 84 recipients received at least one NCBP unit as part of their graft; engraftment was 94% in the entire cohort.[17]

5.0 EFFICACY OF CB TRANSPLANTS FOR SPECIFIC DISEASES

5.1 NCBP CB TRANSPLANTS FOR PATIENTS WITH HEMATOLOGIC MALIGNANCIES

5.1.2 Effect of HLA Match on Relapse

Several analyses have been performed to evaluate incidence of relapse on patients with hematologic malignancies (Acute Myelogenous Leukemia - AML, Acute Lymphoblastic Leukemia - ALL, chronic leukemias and myelodysplastic syndromes – MDS).

The NCBP analyses have shown that the number of mismatched antigens (HLA-A, -B, -DRB1) appears to have no effect on relapse rate. The analysis below is from a cohort of 1066 patients with hematologic malignancies who received single unit CB grafts from the NCBP during the period 1993-2006. Analysis of all patients, as well as subgroups that included patients in remission, or those who engrafted, also failed to demonstrate an association with relapse. [8] (Figures 4, 5, 6 and 7 in section 4, show results from the same analysis).

Table 3. Multivariate analyses of relapse

Variable	n	RR (95% Confidence interval)	*P*
All patients*			
0-MM	56	0.7 (0.4-1.3)	.211
1-MM	352	Reference	
2-MM	588	0.9 (0.6-1.2)	.364
3-MM	65	1.2 (0.7-2.2)	.458
Patients in relapse†			
0-MM	7	1.0 (0.2-4.4)	.977
1-MM	77	Reference	
2-MM	142	0.9 (0.5-1.5)	.693
3-MM	28	1.4 (0.6-3.1)	.394
Patients in remission†			
0-MM	40	0.7 (0.3-1.5)	.359
1-MM	228	Reference	
2-MM	371	0.9 (0.6-1.4)	.662
3-MM	32	1.5 (0.6-3.8)	.411
Patients who engrafted			
0-MM	47	0.7 (0.4-1.4)	.350
1-MM	254	Reference	
2-MM	418	1.0 (0.7-1.4)	.804
3-MM	45	1.7 (0.9-3.4)	.108

MM indicates mismatch; and RR, relative risk.

*Other predictive variables, such as advanced disease, diagnosis of JCML, and younger age (< 10 years), were associated with an increased risk of relapse. TNC, transplant center experience, year of transplant, and patient ethnicity were not predictive of relapse.

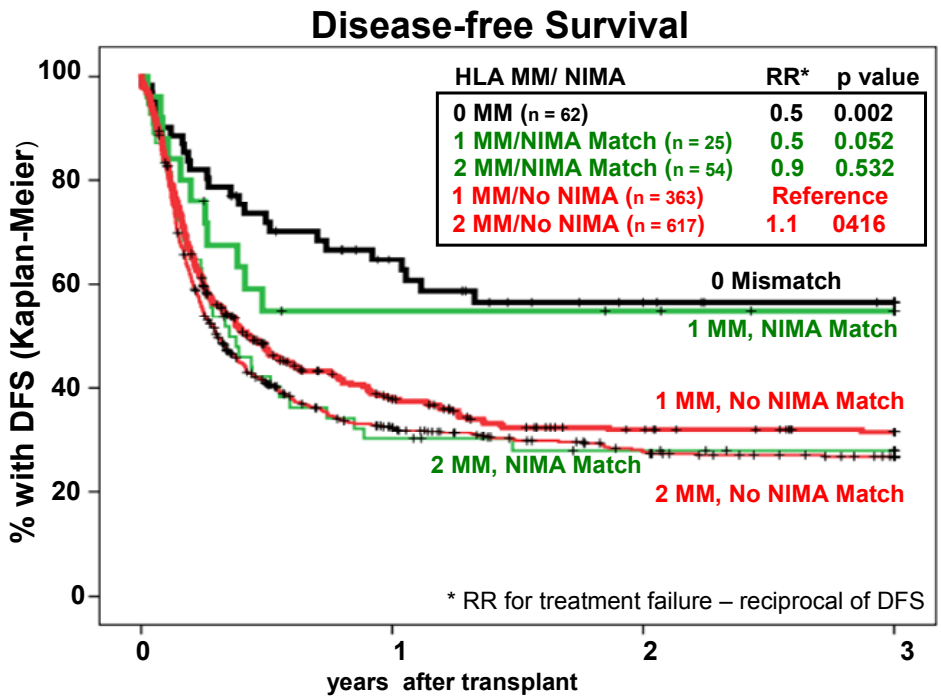
†The subanalyses of those transplanted in relapse or remission excludes those with unknown disease status at time of transplantation and MDS.

5.2.2 Effect of selective HLA mismatches on relapse

Relapse rates after HLA-mismatched, NIMA-matched CB units in patients with hematologic malignancies

However, more detailed analyses have identified specific HLA mismatches to have lower relapse rates. The analysis shown here is from the evaluation of effect of the HLA-mismatched, NIMA matched grafts on disease. Grafts with 1 HLA MM but NIMA match seem to have a lower relapse rate (trend); this was more evident in patients with myelogenous leukemias. [9]

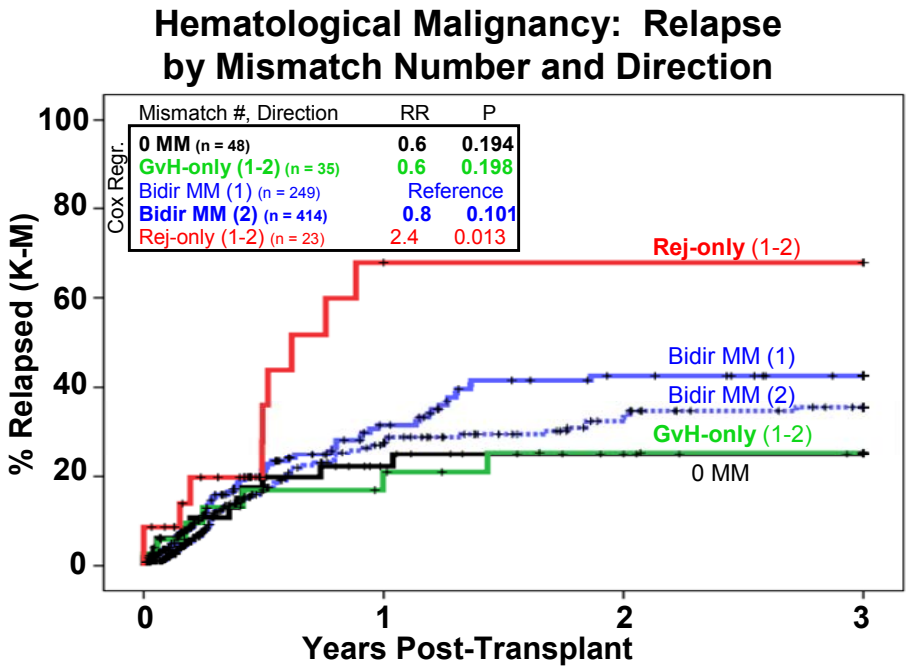
Figure 13: Disease-Free Survival in patients with hematologic malignancies and NIMA matching



Effect of the HLA MM direction on relapse rates in patients with hematologic malignancies

The NCBP analyses have also shown that the direction of HLA MM can affect outcomes (see figure.. section) and in particular, relapse rates.[7]
CB units with 1 MM in the GvHD direction only (GvH-O) had comparable outcomes to those of patients with 0 MM grafts. In contrast, units with rejection - only MM had higher relapse rates (definitions of unidirectional MM are shown in section 4.3)

Figure 14 : Effect of HLA MM Direction on Relapse in patients with hematologic malignancies



5.1.3 Comparison of relapse rates after unrelated CB transplants and unrelated BM/PB transplants

As discussed briefly in section 4.4, NCBP has provided data for three large analyses performed by CIBMTR comparing transplant outcomes of CB units to those of recipients of unrelated bone marrow (BM) or peripheral blood (PB) grafts in pediatric and adult patients with hematologic malignancies.[2,3]

Leukemia free-survival after unrelated CB grafts was comparable to that after 8/8 and 7/8 allele-matched BM in patients> 16 years of age with hematologic malignancies

In the study of pediatric patients (age <16 years) with acute leukemia, outcomes of recipients of unrelated CB grafts (N=503, all NCBP units, all single unit transplants) were compared to those of unrelated BM grafts (N=282).[2] CB grafts were HLA-A, -B antigen and DRB1 allele mismatched to the recipient at 0 (N= 35); 1 (N= 201) or 2 loci (N=267), while BM were either matched at 8/8 alleles (N=116) or 1 allele mismatched (N=166). The 5-year Leukemia-Free Survival after allele matched BM grafts was similar to that of 1- or 2- antigen mismatched CB grafts (Figure 15). Transplants with fully matched CB grafts had even lower leukemia relapse rates.

Note: this CIBMTR study confirmed the results of NCBP analyses showing that HLA matched CB grafts (0 MM) have superior Disease-Free Survival outcomes.

Figure 15: **Probability of leukemia-free survival after BM or CB transplants in patients <16 years of age with hematologic malignancies**

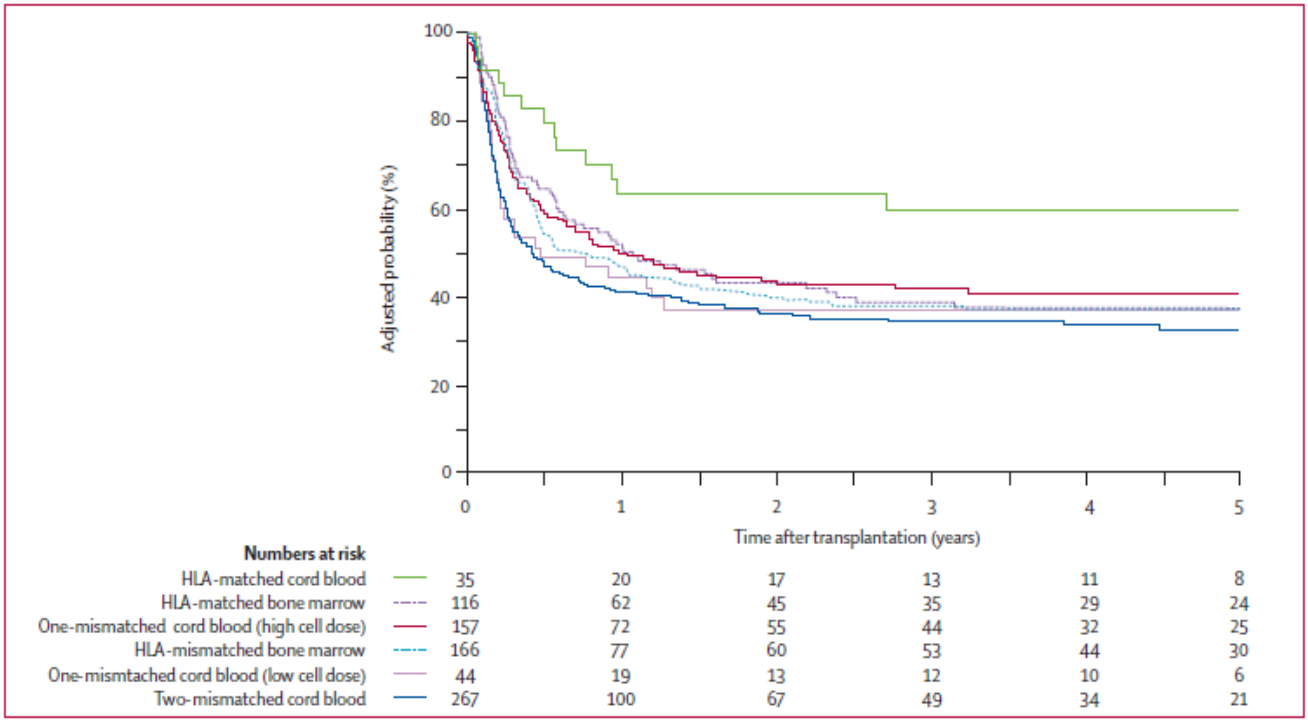


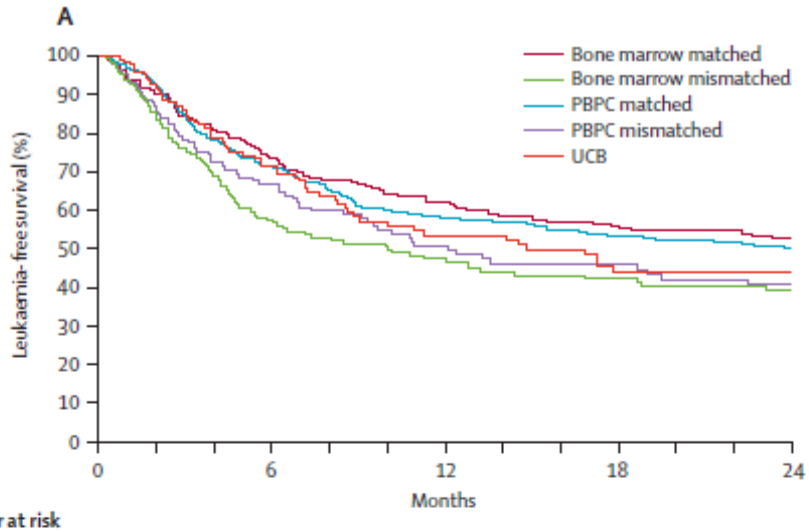
Figure: **Probability of leukaemia-free survival after bone-marrow and cord-blood transplantation adjusted for disease status at transplantation**

Leukemia free-survival after unrelated CB grafts was comparable to that after 8/8 and 7/8 allele-matched BM or PB grafts in patients> 16 years of age with hematologic malignancies

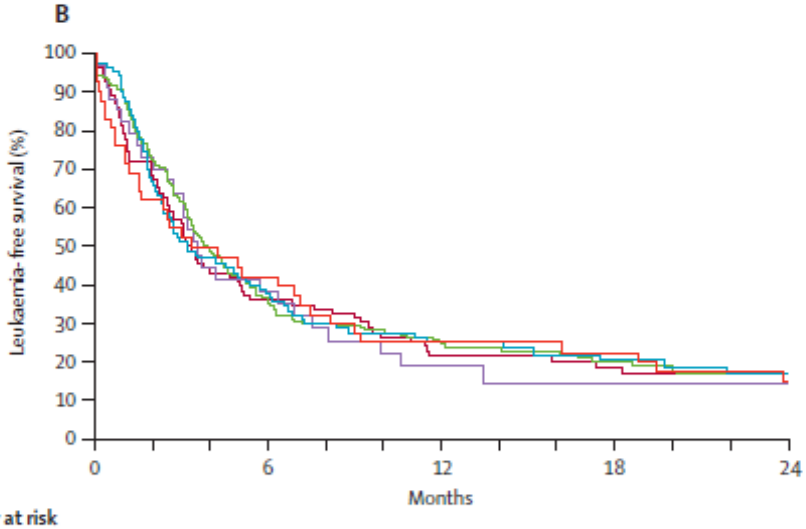
In this CIBMTR analysis, outcomes of recipients of unrelated CB grafts (N=165, all received NCBP CB units, and all had single unit transplants with TNC >2.5x10^7/kg) were compared to those of BM (N=472) and PBSC (N=888) in patients with age > 16 years transplanted during the period 2002-2006 for hematologic malignancies.[3] Of note that CB grafts were HLA-A, -B antigen and DRB1 allele mismatched to the recipient at 0 (N= 10 only), 1 (N= 40) or 2 loci (N=115), while BM and PB were either matched at 8/8 alleles or 1 allele mismatched (7/8 matched).

The analysis concluded that the leukemia relapse was similar in all treatment groups. Relapse rates were higher in patients who were transplanted in complete remission, than those who were not. Leukemia-Free Survival was similar regardless of graft source (Figure 16); this result held when patients were evaluated according to disease status at transplant.

Figure 16: Probability of leukemia-free survival after BM, PB or CB transplants in patients >16 years of age with hematologic malignancies



Number at risk					
8/8 matched bone marrow	246	177	139	99	82
7/8 matched bone marrow	106	69	46	35	31
8/8 matched PBPC	452	316	220	143	112
7/8 matched PBPC	166	89	66	45	36
4/6–6/6 matched UCB	123	77	47	30	24



Number at risk					
8/8 matched bone marrow	86	31	17	13	10
7/8 matched bone marrow	34	12	5	3	3
8/8 matched PBPC	180	63	39	19	14
7/8 matched PBPC	90	34	19	12	8
4/6–6/6 matched UCB	42	17	10	9	6

***Figure 3*: The probabilities of leukaemia-free survival by haemopoietic stem-cell source and donor–recipient HLA match for patients in remission at transplantation (A) and patients who were not in remission at transplantation (B)**

For patients who were in remission at the time of transplantation, the 2-year probability of leukaemia-free survival after transplantation of 4–6/6 HLA-matched umbilical-cord blood (UCB), 8/8 HLA-matched peripheral blood progenitor cells (PBPCs), 8/8 HLA-matched bone marrow, 7/8 HLA-matched PBPCs, and 7/8 HLA-matched bone marrow was 44%, 50%, 52%, 39%, and 41%, respectively. For patients who were not in remission at transplantation, the 2-year probability of leukaemia-free survival after transplantation of 4–6/6 HLA-matched UCB, 8/8 HLA-matched PBPC, 8/8 HLA-matched bone marrow, 7/8 HLA-matched PBPCs, and 7/8 HLA-matched bone marrow was 15%, 17%, 17%, 17%, and 14%, respectively.

5.2 CORD BLOOD TRANSPLANTS FOR METABOLIC DISORDERS (HURLER'S, KRABBE'S AND ADRENOLEUKODYSTROPHY)

The FDA Guidance for Licensure specifies that the indications for the use of licensed CB products (HPC-C) include three inherited metabolic diseases: Hurler's (Mucopoly-saccharidosis type I); Krabbe (Globoid leukodystrophy); ALD (adrenoleukodystrophy). This recommendation was based on the analysis of transplant outcomes provided in the FDA public docket (1997N-0497).

The FDA reviewers of BLA 125397 have requested additional data to evaluate the efficacy of CB transplantation for these diseases.

5.2.1 Brief description of the three metabolic diseases

Inherited metabolic diseases (IMD) are progressive diseases that affect several systems, including the CNS, and lead to significant morbidity, neurologic dysfunction and death in early childhood, in the majority of the affected patients.[16]

Hurler's (MPS I)
Hurler's syndrome, the most severe form of mucopolysacchararidosis, is an autosomal recessive disease caused by a single-gene defect leading to deficiency of the lysosomal enzyme a-L-iduronidase, progressive cellular accumulation of glycosaminoglycans (GAGs) and damage to multiple organs. Patient have rapidly progressive neurocognitive decline and usually die before the age of 10 years.[15] The incidence is approximately 1:100,000 children.

Globoid cell leukodystrophy (Krabbe)
Globoid cell leukodystrophy results from the deficiency of the lysosomal enzyme galactocerebrosidase, characterized by failure of the process of myelination of the central and peripheral nervous system.
The infantile form is characterized by early onset, rapidly progressive neurologic deterioration and death in early childhood.[19] The incidence is also approximately 1:100,000 children.

Adrenoleukodystrophy (ALD)
Adrenoleukodystrophy is an X-linked disorder caused by the deficiency of the ABCD1 gene that encodes a peroxisomal protein membrane. It is associated with the accumulation of very long chain fatty acids (VLCFA). The cerebral form of this condition (cALD) leads to demyelination, rapid neurologic deterioration and death by 10 years of age.[20,21,22]The incidence is higher than the others, approximately 1:18,000 boys.

5.2.2 Rationale for the use of hematopoietic stem cell (HSC) transplants for the treatment of inherited metabolic diseases

CB products provide a source of hematopoietic stem cells for bone marrow reconstitution. Healthy donor-derived cells have the ability to correct the underlying defects in these patients by two mechanisms: a) they can migrate or differentiate to non-hematopoietic tissues (such as microglia in the brain, Kuppfer cells in the liver or alveolar macrophages in the lungs) and provide the enzyme needed in the tissues, and b) they provide "indirect" delivery of the missing enzyme across cellular membranes. This mechanism of "cross-correction", by which, the close proximity of normal (donor) cells producing the enzyme can correct biochemical consequences of enzymatic deficiency in the neighboring cells has been evaluated extensively in cell assays and animal studies.[16]

A different mechanism has been hypothesized for ALD since the defect is a structural protein (peroxisomal); the etiology of the neurologic damage is thought to be related to oxidative damage and/or inflammation and HSC may alter those.

5.2.3 Evaluation of treatment efficacy of HSC in inherited metabolic diseases (Hurler's, Krabbe's and Adrenoleukodystrophy), and relevant medical literature

The NCBP dataset submitted to the FDA (BLA amendment 125397-2) included a total of 223 patients transplanted for inherited metabolic diseases. Among those, 51 were transplanted for Hurler's, 22 for Krabbe and 38 were transplanted for X-linked ALD.
Given the very low incidence of these diseases, we estimate that the patients presented in the NYBC-FDA-IMD dataset represent a significant proportion of <u>all</u> unrelated CB transplant recipients with these diagnoses. For example, a recent analysis combined patients with Hurler's transplanted at Duke University and in Europe (EBMT data); a total of 93 recipients are included, in comparison to 52 patients in the NYBC dataset.[723]

The age at diagnosis, time from diagnosis to transplant, and age at transplant, for these cohorts are shown in Table 1. It is important to note the short times from diagnosis to transplant for some of these patients (interval of only a few weeks) given the risk of rapid neurologic deterioration, and the prompt availability of the CB grafts.

Table 7: Age at diagnosis and transplant for patients with IMDs

Age at diagnosis and at transplant for patients with Hurler's, Krabbe and ALD recipients of CB units manufactured by the NCBP

	Hurler's	Krabbe	ALD
Age at Diagnosis (yrs)			
median	0.90	0.60	7.10
min	birth (2 in utero)	birth (2 in utero)	birth (1 in utero)
max	3.00	4.90	35.00
N (pts with data)	**44**	**19**	**35**
Time Diagnosis - Transplant (yrs)			
median	0.46	0.20	0.60
min	0.19	0.08	0.10
max	2.50	1.60	10.00
N (pts with data)	**44**	**19**	**35**
Age at Transplant (yrs)			
median	1.65	0.65	8.70
min	0.21	0.05	1.70
max	5.60	16.70	43.00
N (all patients)	**53**	**22**	**38**

To evaluate the efficacy of unrelated CB grafts (***as part of allogeneic hematopoietic stem cell transplantation***) in patients with inherited metabolic diseases, particularly lysosomal and peroxisomal diseases, the following aspects need to be evaluated:

- Donor engraftment (conditioning regimen, speed of engraftment, cumulative incidence of engraftment)
- Correction of the enzyme deficiency and the biochemical abnormalities resulting from substrate accumulation
- Prolongation of life – survival, and quality of life
- Multi-organ disease evaluation, with particular emphasis on the amelioration/slower progression of neuro-cognitive dysfunction
- Comparison with other available therapies (if any) - “controls”

Post-transplant information from the recipients of NCBP CB units, as well as published data from transplant centers on all CB recipients were used to evaluate outcomes and are summarized below

Engraftment in patients with inherited metabolic diseases treated with CB grafts

Time to engraftment is usually not long (mean of 18-22 days in different cohorts) given that the majority of the patients are young children and relatively large CB units could be identified.[21,14, 13, 24]

Overall engraftment rates of the transplants performed in the US are approximately 85% using myeloablative regimens.[14] Graft failure has been reported for 8% of patients transplanted in the US [22] and slightly higher rates are seen in Europe [23], probably related to differences in conditioning regimens in the European data. However, with better understanding of the CB graft characteristics that affect outcomes in this group of patients[8] and perhaps closer HLA matching (feasible with larger CB inventories) results are expected to improve.

Further, sustained full donor chimerism has been seen in the majority of CB transplant recipients, in contrast to earlier studies with bone marrow grafts, where mixed chimerism was seen in as high as 30% of the patients.[21, 23]

On the other hand, incidence of GvHD is not negligible (cumulative incidence of acute grade II-IV GvHD of 40% acute Grade III-IV of 10% by day 100 after transplant), and in particular chronic GvHD (incidence in the range of 20%) adds to the morbidity of those patients without any advantageous effect on disease (data from above referenced studies).

Long-term, steady enzyme production

In the patients that engrafted, long-term correction of the enzyme levels in leukocyte samples has been clearly shown (multiple measurements per patient); further, decreased urinary excretion of by-products has also been demonstrated.

Improved survival post-engraftment; quality of life

In the severe forms of the diseases life expectancy with supportive care is very poor. For example, median survival of untreated patients with Hurler's is 6.8 years. In infantile Krabbe disease, 90% of the patients die by age 2 years. Boys with cerebral ALD die by 10 years of age.

The "natural course" of the diseases has been described in relatively small numbers of patients. It is expected that, with the recent development of Registries for specific diseases (e.g. MPS I)[25], more information will become available, particularly regarding presentation at diagnosis, correlation of certain genotypes with phenotype and disease progression, so that treatment options can be selected according to disease prognosis.

In addition to Registries, the implementation of newborn screening programs (e.g., NY State has a screening program for Krabbe) should also assist in collecting more information.

A retrospective comparison of untreated patients with cALD with a similar group that underwent transplantation has been reported for patients with early stage disease. The 5-

year survival probability for the transplanted group was 95%, significantly superior to that of the untreated patients, 54%.[26]

A very important variable affecting the overall post-transplant survival is the clinical status of the patients at the time of the transplant. The Duke series of Hurler patients showed a survival of 77% at 1 year after transplant and 75% at 5 years.[8] For patients with Krabbe disease, survival was excellent (100% at 5 years) for the little babies that were transplanted while asymptomatic, but significantly inferior (43%) for patients with neurologic symptoms.[27]

Similar results were seen in the Minnesota analysis of patients with c-ALD: 5-year survival was 91% for patients with no symptoms of cerebral disease but 66% for those with neurologic dysfunction.[21]

Multi-system disease evaluation and long-term follow-up

This is the most challenging aspect for the evaluation of efficacy of HSC for these patients. Given the rarity of the diseases, the significant clinical variability of the phenotypes, and the lack of large historical data from databases, it is very difficult to evaluate therapy-related changes in organ function and neurologic and cognitive development. Most studies support, however, the stabilization or slower progression of disease in patients that have undergone CB transplantation, particularly in the central nervous system, and outline clinical details and new evaluation tools with the recent experience.

In the Duke analysis of 45 patients with Hurler's disease,[14] all survivors experienced disease stabilization and some gained cognitive skills. Further, while skeletal abnormalities related to the disease remained and patients required frequent orthopedic procedures, encouraging growth data were reported.[14]

In the Duke analysis of 25 patients with Krabbe disease,[19] infants who underwent transplantation with symptomatic disease had high mortality and minimal neurologic benefits. In contrast, asymptomatic newborns with Krabbe (diagnosed because of positive family history) had excellent survival, MRI studies showed normal progression of myelination, they maintained normal vision and hearing and normal cognitive development; however, some degree of gross motor delays has become apparent in several.[19]

The Minnesota analysis of 60 boys with X-linked ALD after HSC (grafts were CB or bone marrow) also highlighted the improved neurologic outcomes of patients transplanted with no neurologic dysfunction.[21]

There is a clear consensus from the clinical studies that patients treated early have higher survival rates and better functional outcomes; a fact that supports the use of CB grafts for these patients as early as possible after establishment of diagnosis and completion of pre-transplant evaluation. It is important to note that, reports from the largest center for unrelated CB transplantation for patients with inherited metabolic diseases show a median time of 35 days from referral to transplantation, underscoring the usefulness of timely availability of CB grafts for these patients.[14]

Other treatment options; are they available and can outcomes be compared to those of HSC?

The other treatment options for these patients are:
a) supportive care, leading to a mean survival below 10 years for patients with severe forms of the diseases,
b) enzyme replacement therapy (ERT), when available, e.g., Hurler's disease,
c) gene therapy as well as other agents are being explored in animal models, but are not available for clinical use

The two studies, that evaluated ERT for Hurler's disease (Laronidase, a licensed product by the FDA) and were submitted in the BLA for the drug, have shown improvement in some patients, particularly those with the attenuated forms of disease (Hurler-Scheie and Scheie), and an overall favorable safety profile compared to HSC (although some infusion adverse reactions have been reported).[28, 29] Further, these studies stress the need for long-term therapy ("life-long"), the variability in responses and the frequent development of antibodies, with unclear effect on efficacy. Most importantly, though, as it is well established that the enzyme does not cross the blood-brain barrier,[30] they showed no effect on neuro-cognitive function.

The FDA medical review[31] notes:" *Given the lack of alternative treatments in a rare disease with severe or fatal consequences, this reviewer recommends approval of laronidase, supported by the evidence of efficacy in the co-primary endpoints and favorable trends in subsets of MPS I in secondary endpoints. Post-marketing studies will be necessary to provide clear understanding of the long-term effects of laronidase in patients with MPS I..:"*

Therefore, the patients treated with ERT do not really represent a "control" group for the severely affected Hurler patients that have undergone HSC. In our opinion, there are no comparable controls for these patients and long-term, systematic, multidisciplinary evaluation is needed to assess the effects of HSC on them.

For Krabbe disease, the only effective therapy is HSC, there is no enzyme replacement.[16] Similarly, no specific therapy exists for patients with cALD after development of symptoms.[20, 21]

5.2.4 Recommendations for the treatment of inherited metabolic diseases from the literature

While for some physicians, HSC is the "standard of care" for patients with the severe forms of these metabolic diseases particularly before the onset of symptoms,[15] others are more hesitant. Clearly more precise evaluation tools are needed so that prognosis can be accurately assessed and risks can be evaluated.

An international panel of experts has provided the following recommendations for Hurler's:[32]

"HSC appears to be the only currently available clinical treatment that offers the ability to induce long-term correction of the metabolic abnormalities, prolong survival and ameliorate/preserve or stabilize the neurocognitive function. The patient's age (> 2years or ≤ 2 years), predicted phenotype, and development quotient help define the risk/benefit profile for hematopoietic stem cell transplantation (higher risk, but can preserve central nervous system function) versus enzyme replacement therapy (low risk, but the enzyme cannot cross the blood brain barrier). [28 ,30, 32]
While the age cut-off (2 years) has been challenged by other investigators, disease and performance criteria are accepted in algorithms of treatment approaches and additional investigations are needed to further define those.

Further, it is clearly noted by the above expert panel that "enzyme replacement is not curative".[28, 30]

Regarding Krabbe disease, there is consensus that no other therapy is currently available.[33] Further, asympomatic patients seem to have excellent survival rates after HSC. It should be noted, though, that even successfully transplanted patients may show some deterioration in time and even patients transplanted with pre-symptomatic disease have shown some neurologic and other deficits.[33]
One of the problems is accurately assessing prognosis, since neither the enzyme activity nor the mutation can predict the severity of the disease. To improve that, a recent study implementing clinical criteria including neurodevelopmental evaluation to support the construction of treatment algorithms is very helpful.[34]

Similar concerns regarding more accurate assessment of prognosis exist for cALD. In some studies, characteristic leukodystrophic changes in MRI studies (Loes scores) which typically precede clinical symptoms appear, to be a strong predictor of outcome.[35]

5.2.5 Summary of clinical information for IMDs

The above diseases

- result from single recessive gene defects
- the enzymatic deficiency leads to decreased enzyme levels in the blood and accumulation of abnormal substrate in the tissues (that is responsible, in part, for the disease-specific phenotype) that can be measured and followed over time; correction of the metabolic abnormalities are seen after successful engraftment of donor cells [2136]
- their incidence is rare, so it is not feasible to study large numbers of patients
- there is significant variability in disease progression; further, genotype does not always predict phenotype
- they lead to rapid, progressive neurologic deterioration and death in early childhood
- <u>there is no other clinical treatment that can change the neurologic outcomes</u>
- clearly HSC therapy with unrelated CB grafts can provide benefit in some patients, stabilize, ameliorate or prevent neurologic damage and perhaps reverse some of the phenotype if treatment is administered early in the course of the disease. However, morbidity and mortality are serious considerations.

- new tools are being developed to predict prognosis better and develop more specific treatment algorithms

  We propose to have these diseases as indications for licensed CB products and continue the multi-system, long-term evaluation of patient outcomes with post-marketing studies, in collaboration with the transplant centers and CIBMTR.

6.0 SUMMARY OF NCBP CLINICAL DATA – IMPLICATIONS FOR THE INDICATIONS FOR LICENSED HPC-C PRODUCTS

Historically, NCBP has viewed CB as a source of hematopoietic stem cells, alternative to BM or PB, for all patients requiring BM reconstitution independently of the underlying diagnosis. To that effect the IND title was "Placental Cord Blood for BM reconstitution" (BB-IND 6637, granted in 1996).
The NCBP clinical data were submitted to the FDA public docket (1997N-0497) and these, along with other banks' data were the basis of the FDA Guidance for Licensure.

For the BLA, NCBP elected to apply for all the indications that were recommended in the FDA Guidance. We have summarized in the previous sections the clinical results of 3532 patients and 3946 NCBP CB units, in selected outcomes analyses. Of note, outcomes are available for other, more rare, diseases, since 4% of the recipients had diseases not included in the FDA indications; these are not presented here. The Table below shows the diagnoses of patients included in the NCBP Outcomes Database (Table 3, included in section 1.3).

The efficacy of unrelated CB grafts, as part of allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies is accepted by the transplant community. Further, the results of the CIBMTR comparison studies with NCBP indicate that the Disease-Free Survival after unrelated matched BM or CB (at appropriate cell doses) is similar, in both pediatric and adult patients. Moreover, very encouraging preliminary results regarding improved Leukemia-Free Survival are reported after double unit CB grafts (these are transplant center studies with some -but not all- patients receiving NCBP products).

We have also presented outcomes in non-malignant diseases, where treatment efficacy is more complex to evaluate, for the reasons described. In those cases, correction of the metabolic/enzymatic abnormalities is achieved in the blood of the majority of the patients, however, reversal of the underlying pathology and disease phenotype may or may not follow, especially if the disease has progressed.
However, clinical outcomes reported by transplant centers (see references in section 5.2) clearly support use of unrelated CB grafts for <u>some</u> (selected) patients and ongoing, as well as post-marketing studies will be important in defining the selection criteria. In our opinion, this is also needed for other diseases, not currently included in the FDA recommended indications, but for which CB transplants are being performed successfully, such as Sickle Cell disease or Osteopetrosis.

Considering these points, and current clinical practices, NCBP would propose a broader definition of indications, not strictly by disease entities but by pathophysiologic mechanisms, such as reconstitution of the hematopoietic and immune system, correction of genetic structural or functional deficits of specific cellular enzymes and hemoglobin.

Such broader definition would encourage the production and use of licensed products, meeting all FDA requirements, and under the supervision of the FDA, for a large number of recipients and a variety of diseases. We are convinced that both, patient safety and clinical outcomes, will greatly benefit from the transplantation of FDA-licensed products.

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Patient characteristics: Diagnosis and Diagnosis groups

Diagnosis Group	N	% (of total)	(% of disease group)
Leukemia	**2177**	**61.6%**	
ANLL	960		44.1%
ALL	916		42.1%
Chronic leukemias	301		13.8%
MDS	**192**	**5.4%**	
Lymphoma	**228**	**6.5%**	
BM failure	**246**	**7.0%**	
Histiocytosis	**87**	**2.5%**	
Metabolic/storage disease	**223**	**6.3%**	
Krabbe	22		9.9%
ALD	38		17.0%
Hurler's	51		22.9%
immunodeficiency	**212**	**6.0%**	
Hemoglobinopathy	**56**	**1.6%**	
SCD	23		41.1%
thalassemia	33		58.9%
platelet disorders	**5**	**0.1%**	
neutrophil disorders	**32**	**0.9%**	
other malignancy (multiple myeloma, neuroblastoma	**29**	**0.8%**	
other	**3**	**0.1%**	
(unknown)	32	0.9%	
Total	***3532***		

Diagnosis Group		
malignant	**2621**	**74.2%**
non-malignant	**869**	**24.6%**
(unknown)	42	1.2%
Total	**3532**	

(for this analysis, patients with two transplants are counted at first transplant only)

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Overview of CIBMTR Cord Blood Data Collection:

Overview of CIBMTR

The Center for International Blood and Marrow Transplant Research (CIBMTR) collaborates with the worldwide scientific community to advance hematopoietic cell transplantation (HCT) and cellular therapy research. As a combined research program of the Medical College of Wisconsin (MCW) and the National Marrow Donor Program (NMDP), the CIBMTR facilitates important clinical research to increase survival and enrich the quality of life for thousands of HCT patients. CIBMTR research arises from a base of collaborative scientific and statistical expertise, a large network of transplant centers, and a clinical database of more than 300,000 transplant recipients. Information from the database, and the support provided by the CIBMTR Statistical Center to analyze it, have led to successful completion of hundreds of studies that have had significant impact on clinical practice.

Stem Cell Therapeutic Outcomes Database (SCTOD)

The Stem Cell Therapeutic and Research Act of 2005 (Public Law 109-129) established the C.W. Bill Young Cell Transplantation Program (the Program). The Program was designed to make information about HCT available to patients, families, health care professionals and the public; to provide better processes for identifying unrelated matched marrow and peripheral blood stem cell donors and cord blood units through one electronic system; to increase the numbers of unrelated adult volunteer marrow donors and cord blood units that are available; and to expand research to improve patient outcomes. Under this Program, the Stem Cell Therapeutic Outcomes Database (SCTOD), administered by the CIBMTR, collects, analyzes and reports on outcomes for all allogeneic transplants and on other therapeutic uses of blood stem cells performed in the United States or using a product obtained in the United States. The emphasis of the SCTOD is on collecting a basic set of data that enables analyses of Program use, center-specific outcomes, donor registry and cord blood inventory size, and patient access to HCT. The CIBMTR is in a unique position to use the data collected for recipients of cord blood transplantation to provide clinical outcome data to originating cord blood banks for analysis and quality assurance activities.

The unique position of the CIBMTR brings significant value to transplant centers who provide data, cord blood banks who use data for quality assurance analytics, and the scientific community whose research addresses important clinical decision-making for transplant recipients. Using the centralized platform of the CIBMTR brings value to cord banks by streamlining data collection to a single, cooperatively developed mechanism which collects standardized data elements with quality monitoring in the setting of required reporting. Banks receiving outcome reports can focus on analysis of the data for the benefit of their manufacturing processes, without being burdened by development of their own data collection processes. Transplant centers who use cord blood products for hematopoietic transplantation benefit from a universal data collection system and processes that avoid redundant data submission that is burdensome and error-prone, provide validation closer to the source of the

data, and are accompanied by education efforts targeted to improving data quality. Finally, researchers benefit from a consolidated source of high-quality data with adequate numbers to address important research questions beyond those that can be answered using data from a single bank or transplant center, and in the larger context of hematopoietic cell transplantation regardless of cell source.

Description of CIBMTR data collection systems, forms and timelines, CPI and auditing

Centers inform the CIBMTR about their HCT recipients using FormsNet™2, a web-based application, which generates a unique ID for each recipient. This unique ID is then used as a linked identifier for all subsequent data submission, and to connect the recipients' outcomes data to the donor information as provided by the transplant center. The forms have an assigned due date based on the recipient's HCT date. Timely submission of data to CIBMTR is required for completion of cord blood outcomes reports. CIBMTR has established criteria for submitting the forms. The system that monitors how well these criteria are met is called Continuous Process Improvement (CPI). Further details can be found in the CIBMTR Data Management Manual (Section 17). To be fully compliant with CPI, a transplant center must submit at least 90% of the forms due within the time periods established for each form during a particular trimester. A brief summary of data collected, forms upon which it is collected, and timelines for submission is shown in the following tables.

Pre-HSCT Forms	Date Expected	90% submitted within
Pre-TED Form* Planned Preparative Regimen GVHD Prophylaxis Donor/CBU source Recipient Comorbidities Indication (disease) for transplant Disease status at transplant	14 days prior to HCT	By HCT date
Baseline Form Detailed Preparative Regimen Recipient Coexisting conditions Infection data (when applicable) Pre-HCT Disease Form(s) Detailed diagnosis information Disease status at HCT IDM and HLA Forms* Donor/CBU/Maternal IDM Test Results Donor/CBU HLA typing	HCT date	60 days of HCT date
Infusion Form* Shipment Details Thawing Details Manipulation Details Post-thaw and infusion analysis Sterility and CFU results Infusion Details Infusion AE Summary†	HCT date	60 days of HCT date

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Post-HSCT Forms	Date Expected	90 % submitted within
100 day Post-HCT Data* Survival Engraftment Chimerism GVHD Post-HCT Disease Form(s) Disease status at HCT Therapy given since last report Infection data (when applicable)	Day 100 post HCT	120 days of due date
Six Months to Two Years Post-HCT Data* Survival Engraftment Chimerism GVHD Post-HCT Disease Form(s) Disease status at HCT Therapy given since last report Infection data (when applicable)	6 months, 1 year, and 2 years on HCT anniversary	90 days of due date
Yearly Follow-up (>2 years) Post-HCT* Data Survival Engraftment Chimerism GVHD Post-HCT Disease Form(s) Disease status at HCT Therapy given since last report Infection data (when applicable)	Starting year 3, annually on HCT anniversary	45 days of due date

*Forms indicated with an asterisk represent the minimum data collected during the time interval for recipients of cord blood HCT in the United States. In some cases, additional data is collected for recipients whose data is reported to the CIBMTR on the Comprehensive Report Form (CRF) where more details of disease and transplant complications are collected.

†Data regarding infusion-related adverse events is collected in summary fashion on the infusion form for validation and research purposes, and is not meant to replace real-time AE reporting by HCT centers to the CBCC or the originating bank or registry.

CPI standing for all HCT centers is reviewed on a trimester basis. Ninety percent of the forms expected in the preceding trimesters must be completed to be considered compliant for CPI. If a transplant center is continuously non-compliant, they will not be allowed to participate in CIBMTR studies and their access to unrelated donors through NMDP will be restricted. Historically, this program has been very successful for NMDP, resulting in timely forms submission.

CIBMTR also has an on-site data audit program. Each domestic and international center is audited once within a four-year audit cycle. All TED and CRF level data are subject to the data audit. Critical data fields are audited for each recipient selected for audit. These are data fields that are included in the majority of outcomes analyses.

Description of Cord blood data collection, processes, validation and queries, and data quality initiatives

CIBMTR is committed to collecting high quality data regarding all HCT procedures and outcomes reported to the outcomes database. In addition, the CIBMTR is committed to using the data collected for cord blood transplantation to generate reports about outcomes to the cord blood banking community for units provided by the respective banks. The CIBMTR has indicated to transplant centers that timely submission of follow-up forms for cord blood transplants are a high priority. In addition to the standard CPI and auditing programs, CIBMTR staff generate a report of forms due and completed twice monthly for cord blood transplants to review progress of data submission by HCT centers. Similar reports are used to determine if there are any gaps in reporting of cord blood transplants performed compared to those reported to CIBMTR. Using these reports, CIBMTR staff work with the transplant centers to resolve discrepancies and prompt data submission. Additionally, for each bank that provides a list of units shipped for HCT, CIBMTR staff reconcile this list with the cases reported and request reports for any cases that have not yet been completed.

In addition to requesting completion of forms, CIBMTR staff send proactive queries to transplant centers to resolve data validation errors for completed forms. These queries are based on a set of validation ranges specific to Cord Blood HCT for the data fields considered critical to the cord blood banking and research community, as determined by the Cord Blood Data Working Group. Cord Blood Banks are also encouraged to query CIBMTR for any data found in the Cord Blood Outcomes Reports that they suspect may not be accurate.

Examples of queries sent to HCT centers include:

- Missing or invalid Cord Blood Unit IDs or source (CBB)
- Verification of additives reported, manipulations, shipment, and thawing details
- Verification of cell dose (by reviewing stem cell thaw records), post-thaw cell recovery
- Review of chimerism data reported

Responses to queries are generally incorporated into the Database immediately, and become available in the next Cord Blood Outcomes report. Issues found with these queries are also used to inform the development of CIBMTR training efforts directed at the transplant centers. Live sessions, instruction manuals and supplements, webinars, and one-on-one sessions have all been used to train transplant center staff about these issues.

Description of Cord blood outcomes reports for banks

Cord Blood Outcomes data are sent regularly to banks by CIBMTR staff at the frequency determined by the Cord Blood Data Working Group (currently monthly). Data contained in reports are usually generated from the database less than a week prior to being sent to banks.

Banks are notified of the last “refresh” date of the data when the reports are sent. Outcomes reports are provided as a Microsoft Excel format, with tabs dividing each section of the report. Additionally, a comparison report is generated for each bank, highlighting additions or changes to the report since the previous version, allowing banks to quickly identify updates.

The variables in the Cord Blood Outcomes Report (Blinded sample attached) include:

Baseline –

- Recipient characteristics (race, ethnicity, ABO and Rh type, height, weight, diagnosis date)
- CBU Characteristics (race, ethnicity, ABO and Rh type)
- Recipient Research Consent status

Infusion –

- Products Infused
- Recipient Age, diagnosis, disease stage, performance score at conditioning
- Recipient Survival status and COD

Conditioning / Post-transplant

- Recipient Preparative Regimen Details
- Engraftment Details (neutrophils and platelets)
- GVHD Details

Product –

- Pre-freeze CBU Characteristics (as available through the CBCC)
- Post-thaw TNC, CD34 & Viability
- Post-thaw Recovery
- Wash indicator
- Infused TNC, CD34 & Viability
- Manipulation Details
- Thaw and Infusion AE Indicators
- CFU Counts
- Sterility Cultures
- Indication of additional products

Single Product Chimerism Test Results
Multiple Product Chimerism Test Results

The data collected by the CIBMTR that are most critical to Cord Blood Banks as they consider product quality assurance is information on post thaw characteristics of the graft product (post thaw cell counts and viability, post-thaw recovery) and time to hematopoietic recovery (as measured by time to neutrophil and platelet engraftment). Chimerism data is collected for recipients through 2 years after HCT, and is particularly relevant for recipients of multiple cord products. These data are collected on all HCT recipients on post transplant follow-up forms, or on the infusion form (Form 2006) at the time intervals described above. These very critical data fields also receive focused attention for validation when CIBMTR generates reports to banks as described above. Although real-time data collection of adverse events related to cord blood transplantation is not the purpose of the observational data systems of the CIBMTR, summary information about infusion-related adverse events is collected by the CIBMTR and is provided to banks as a tool for verification of adverse event reporting associated with cord blood units.

CIBMTR® CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

Description of Cord Blood Data Working Group (CBDWG) and meeting needs of cord banks for data collection and outcomes assessment.

In order to maximize the usefulness of data collected by the CIBMTR for the cord blood community, the Cord Blood Data Working Group was formed in early 2009. This group includes representatives from cord banks, transplant centers, researchers, relevant CIBMTR staff and HRSA staff. The group meets on a monthly or every other month schedule, by teleconference, and discusses issues relevant to the SCTOD and cord blood outcomes reporting. Topics include content and format of the cord blood outcomes reports, training for transplant centers, revisions to CIBMTR data collection instruments, bank licensure and other issues of importance to the cord blood community. The CBDWG helps to ensure that the data contained in the Cord Blood Outcomes Reports are sufficient for banks to analyze for quality assurance purposes.

Summary

The CIBMTR is uniquely positioned and committed to use clinical outcome data collected in the context of the SCTOD to meet the needs of the cord blood transplant community. Data collection from HCT centers performing allogeneic HCT using cord blood products is required as part of the CW Bill Young Program. CIBMTR has developed data collection instruments, electronic systems and processes to acquire the relevant data. Further, these systems are supported by a CPI and auditing system, as well as data quality initiatives specific to cord blood reporting. CIBMTR has actively engaged the HCT community in training efforts to describe the data submission requirements and their importance to the cord blood banking system and future availability of high-quality cord blood units, as well as to provide instruction regarding completion of CIBMTR data collection instruments. The centralized system is designed to collect and report data in a standardized way that avoids redundancy, minimizes burden and provides high quality data and reports for the stakeholder groups. CIBMTR has also actively engaged the cord blood banking community in the revision of the Cord Blood Outcomes Reports to maximize their usefulness. Collectively these efforts yield high quality Cord Blood Outcomes Reports on a regular frequency that contain the core of clinical outcome information essential to cord blood banks for quality assurance efforts.

New York Blood Center
National Cord Blood Program
45-01 Vernon Blvd., Long Island City, NY 11101
Ph: (718)-706-5211

NATIONAL CORD BLOOD PROGRAM

Procedure for Cord Blood Units Thawing and Preparation for Administration

BACKGROUND

Optimally, thawing successfully cryopreserved cells requires removal of the intracellular cryopreservative and its replacement by water in a way that returns nucleated cells to normo-osmolality, with high viability and preserved function. A practical method for thawing, diluting and removing the DMSO utilized by the New York Blood Center (NYBC) National Cord Blood Program (NCBP) since its inception, was reported in 1995 (Ref. 1). Recently, a simplified method has become appealing, that eliminates the centrifugation step and removal of the DMSO-based cryoprotectant (Ref. 2). This variant (thawing with reconstitution) may be preferred because it is shorter and it avoids any loss of cells into the removed supernatant, thereby increasing the cell dose for the patient (Ref. 3). This cell loss with the "wash" method can be important, although it can be obviated by the centrifugal recovery of the cells lost into supernatant-1. Not removing the supernatant, results in the infusion of gram quantities of DMSO, relatively quickly, into the recipient's vascular system.

The thawing procedure includes three parts: thawing the frozen HPC-C product, reconstitution (diluting the thawed product), and washing (removal of cryoprotectant DMSO)

1. PROCEDURE

1.1. With the exception of the cord blood units (CBUs) frozen in the beginning of the NYBC Program (1993-1994), as whole, unmanipulated blood containing 10% DMSO, in Delmed freezing bags (CharterMed), all NCBP CBUs are partially red cell- and plasma-depleted, cryoprotected by admixing 50% DMSO to yield a final concentration of 10% (total volume = 25 mL) and stored either in Cryocyte bags (Baxter) (Figure 1) or, since 1999, in compact, three-dimensional freezing bags (Figure 2) designed for freezing in the BioArchive System. The name of the product contained in these freezing bags is Hematopoietic Progenitor Cells, Cord (HPC-C).

Figure 1.

Figure 2.

1.2. For both types of freezing bags with NCBP HPC-C product (Figures 1 and 2), the procedure to restore the osmolarity of the blood cell suspension and either remove the supernatant with DMSO or simply dilute the thawed HPC-C, is assisted by a sterile, empty, transplant bag set "Cell Wash/Infusion Bag Set" (Figure 3), which is included with this shipment (Pall Medsep re-order code 791-03), and is designed with two spike tubes to drain both compartments of the later freezing bag.

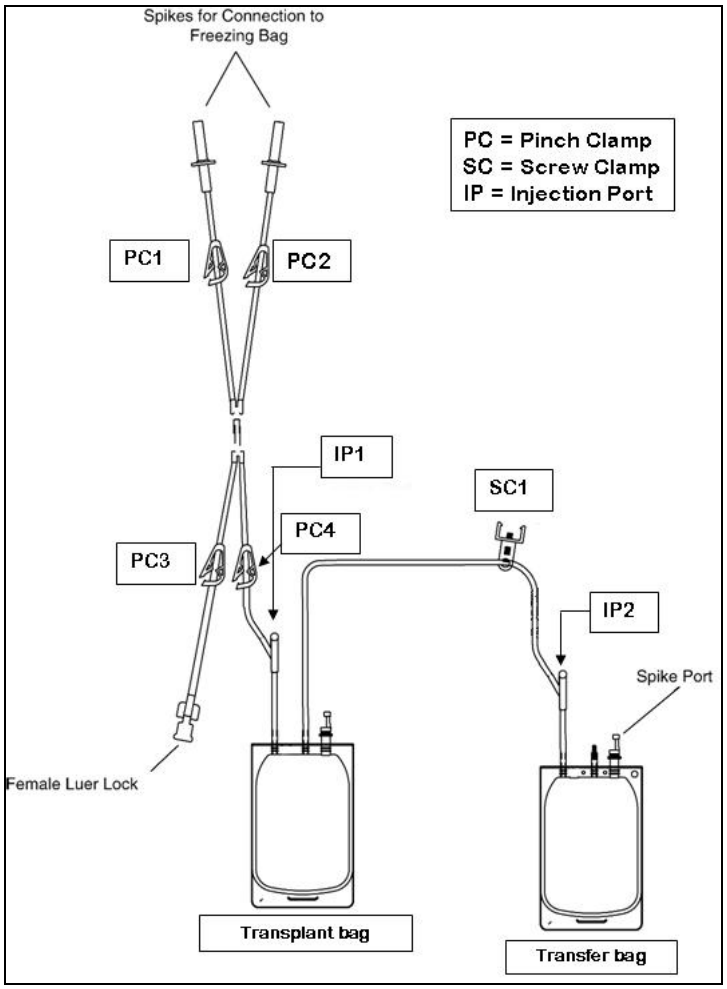

Figure 3. Cell Wash/Infusion Bag Set
(Transplant Set)

1.3. For HPC-C units frozen in Delmed bags, due to the large volume of frozen blood, thawing with dilution and removal (or not) of DMSO should be accomplished using either 300 or 600 mL transfer pack containers.

2. MATERIALS

2.1. Cord Blood Unit

2.1.1. Frozen CB unit (identified as described in section 3) in a steel canister enclosed in a foam sleeve and kept at a temperature below -150° C, either inside the container used for shipping (Dry-Shipper) or in a Liquid Nitrogen (LN_2)-cooled storage device at the Transplant Center (recommended).

2.2. Equipment

2.2.1. Biological Safety Cabinet (BSC)

2.2.2. Refrigerated blood bank centrifuge

2.2.3. Plasma expressor (Baxter 4R4414 or similar)

2.2.4. Digital balance

2.2.5. Tube sealer compatible with PVC plastic

2.2.6. Automated cell counter

2.2.7. Microscope and chamber for determining cell count and viability (optional)

2.2.8. Waterbath (4 liters or more)

2.2.9. Canister opening tool (THERMOGENESIS CORP.)

2.3. Reagents

2.3.1. Albumin (5% human USP) (CHL Behring)

2.3.2. 10% Gentran 40 (Dextran 40), USP (Hospira)

2.3.3. Bacterial culture bottles (aerobic and anaerobic)

2.4. Supplies

2.4.1. Cell Wash/Infusion Bag Set (Transplant Set) (Pall Medical 791-03) (included with NCBP frozen product)

2.4.2. Sterile Disposable Syringes: 3 mL, 30 mL and 60 mL (Becton-Dickinson)

2.4.3. 18 gauge injection needles

2.4.4. Sterile gloves

2.4.5. Hemostats

2.4.6. Sterile small plastic zipper-lock bags

2.4.7. Alcohol prep pads

2.4.8. Iodine swab sticks

2.4.9. Sampling site couplers

2.4.10. Tubes for cell counts, progenitor assays (optional)

2.4.11. Protective cryogloves

2.4.12. Transfer pack container 300 or 600 mL (Fenwal)

2.4.13. CB thawing procedure

3. VERIFICATION OF UNIT'S IDENTITY

3.1. The CB unit is shipped in a canister which is contained in an insulating foam sleeve.

3.2. The bar-coded ID label of the CB unit, affixed to the canister, is visible through the open side of the canister sleeve (Figure 4).

Figure 4.

3.3. Check the CB unit ID label to confirm its identity with the ID of the expected unit <u>as soon as it is received.</u>

3.4. Wearing protective cryogloves, transfer the CB unit from the Dry-Shipper to the vapor phase of a LN_2 storage tank.

3.5. Use the TG canister opening tool to pry canister open at top and bottom, as shown below in Figures 5 and 6.

3.6. Work carefully to avoid damaging the frozen plastic CB bag.

3.7. Check the bar-coded label on the unit against your records to verify that the bar-coded and visually-readable printed number absolutely conform to:

3.7.1. the information previously provided,

3.7.2. the documentation included with the unit.

3.8. Document this check on the NCBP "Unit Receipt Form" document.

Figure 5.

Figure 6.

NOTE: **If there is <u>any</u> error or ambiguity with regard to the unit ID, close the canister and keep the unit at LN_2 temperature. <u>Immediately advise the National Cord Blood Program at NYBC and the transplant physician</u>. Do not proceed until the problem is resolved. If your LN_2 storage tanks have no space to store the unit in its canister and insulated sleeve, add LN_2 to the NCBP dry-shipper to maintain the unit frozen until a completely satisfactory determination is made.**

4. METHOD

4.1. Preparation of thawing solutions

4.1.1. Prepare the thawing solution (also called reconstitution solution) at room temperature, mixing equal volumes of 10% Dextran 40 and 5% human albumin, in a biological safety cabinet. The final

concentration in thawing solution is 5% Dextran 40 and 2.5% human albumin.

4.1.2. Attach an 18 gauge needle to a 30 cc syringe. Draw approx. 12.5 mL of 10% Dextran 40 and approx. 12.5 mL of 5% human albumin into the syringe. This syringe is to be used for diluting the cell suspension after thawing (for a volume of frozen blood of approx. 25 mL).

4.1.3. Fit 18 gauge needles to three 60 mL syringes. Draw 30 mL of 10% Dextran 40 and 30 mL of 5% human albumin into each syringe. Two of these 60 mL syringes will be used in steps 4.4.2.11. and 4.4.2.13. of this procedure and the third one in step 4.5.9.

4.1.4. Alternatively, prepare the thawing solution in a 300-mL transfer bag by adding, using syringes, 150 mL 10% Dextran 40 and 150 mL 5% albumin.

4.2. **Thawing the unit**

4.2.1. Wearing protective cryogloves, remove the canister with the unit from the LN_2 container. Keep the canister in the vapor phase, just above the surface of the LN_2 for 5-10 minutes before proceeding.

NOTE: **If two units are stored in the LN_2 container at the same time, open one canister at a time with the TG canister opening tool as described in 3.5. Carefully check the ID number on the labels attached to the canister and the unit, respectively. Close the canister and leave it in the vapor phase for 5-10 min. before proceeding.**

4.2.2. Open canister with the TG canister opening tool as described in 3.5. (Figures 5 and 6).

4.2.3. Work carefully to avoid damaging the frozen plastic CB bag. Remember that plastic at this temperature is very brittle and breaks easily.

4.2.3.1. ***To recover the product (Hematopoietic Progenitor Cells, Cord/ HPC-C) from ruptured freezing bags follow the procedure described in section 6***.

4.2.4. Examine the bag for breaks or cracks and document this inspection on the appropriate form.

4.2.5. Remove any segment attached to the product bag before thawing, if still present. Store the segment in LN_2 before thawing and testing.

4.2.6. Remove the unit from the canister.

CAUTION: Do not handle plastic bags at liquid nitrogen temperature with the thongs intended for metal canisters!
Do not allow unit or tubing to bend or it will crack!

4.2.7. Put the CB unit inside a zipper-locked plastic bag, let the air out and close the bag. Place the bag with the CB unit in a warm waterbath at approximately 38°C.

4.2.8. To accelerate and homogenize thawing, carefully agitate the unit in the water and gently knead its contents.

NOTE: ***Inspect and watch for leaks*.**

Plastic bags for frozen blood may have pin-holes, other defects or develop leaks. It is most important to prevent blood losses and/or bacterial contamination caused by leaks.

If blood leaks out into the zipper-locked bag, find the site of the break in the freezing bag and position the unit so as to prevent further escape of blood. While maintaining it in that position, finish thawing the unit. Proceed to 4.2.9. Refer to section 6 - Emergency product recovery.

4.2.9. As soon as the bag's contents become slushy, remove the bag from the waterbath and place it inside a biological safety cabinet.

4.3. **Connecting the Freezing Bag to the Transplant Set**

NOTE: The following procedure must be done in a biological safety cabinet.

4.3.1. Close all clamps on the Transplant Set.

4.3.2. Remove the unit from the zipper-locked bag.

4.3.3. Disinfect the covers of both ports of the freezing bag with iodine.

4.3.4. Using a clean and disinfected scissors, cut off the hermetically sealed covers of the freezing bag's spike ports (Figure 7).

Figure 7.

4.3.5. Disinfect the cut surfaces of the spike port area of the freezing bag using iodine swab sticks (Figure 8).

Figure 8.

4.3.6. Insert the spikes of the transplant set into the ports of the freezing bag.

NOTE: Label the transplant bag with the CB unit ID number and the name of the recipient, or according to local standard practice.

4.4. **Reconstitute (dilute) the thawed Cord Blood**

4.4.1. The amount of thawing stock solution used for the volume reduced units (partial red blood cell depleted) is at least 5 times the volume of the frozen blood including the cryoprotectant.

4.4.1.1. For example, 25 mL units are diluted to 170 mL total and thus, a volume of 145 mL of thawing solution is required to make the final volume of 170 mL in a transplant bag.

4.4.1.2. Note that if the cryoprotected volume of the unit is larger than 25 mL, a larger transfer pack container will be required (300 or 600 mL).

4.4.2. Add first a volume of thawing solution equal to the volume of thawed blood (1:1 ratio).

4.4.2.1. Attach the 30 cc syringe with the 25 mL thawing solution to the female luer lock of the transplant set.

4.4.2.2. Open PC-1, PC-2 & PC-3 (see Figure 3 in page 3) and then slowly introduce half (~12.5 mL) of the thawing solution to the 25 mL product in the freezing bag while mixing the fluids in the bag using an orbital rotator.

4.4.2.3. Rinse well to remove cells from the bag's ports.

4.4.2.4. Close PC-3, open PC-4 and drain the contents from the freezing bag into the transplant bag.

4.4.2.5. Close PC-1 and PC-2 and open PC-3.

4.4.2.6. Add slowly the remaining thawing solution (~12.5 mL) to the transplant bag while mixing the fluids in the bag.

4.4.2.7. Close PC-3.

4.4.2.8. Allow approx. 5 minutes for equilibration.

4.4.2.9. Open PC-1 and PC-2 and pass the diluted CB back and forth between the transplant bag and the freezing bag in order to more completely wash all cells out of the freezing bag and into the transplant bag.

4.4.2.10. Close PC-1 and PC-2.

4.4.2.11. Attach a syringe with 60 mL dextran-albumin solution to the luer lock. Open PC-3.

4.4.2.12. Transfer the 60 mL solution to the diluted CB in the transplant bag while mixing the fluids in the bag.

4.4.2.13. Repeat with a second 60 mL syringe. The final volume is now approximately 170 mL (50 mL diluted CB and 120 mL dextran 40-albumin solution).

4.4.2.14. Close PC-3 and open PC-1 and PC-2.

4.4.2.15. Pass the reconstituted CB back and forth between the transplant bag and the freezing bag in order to wash all cells out of the freezing bag and into the transplant bag completely.

4.4.2.16. Close PC-4.

4.4.2.17. Seal the Transplant Bag Set tubing between PC-4 and IP-1 and cut through seal to separate the transplant bag from the freezing bag.

4.4.2.18. Discard the freezing bag, the luer lock and the connecting tubing.

4.4.2.19. The reconstituted product can be used for infusion into a patient with or without the additional step of DMSO removal (section 4.5 below).

4.4.2.20. The recommended expiration time of the reconstituted but not washed product is four hours either at room temperature or at 4˚C from the time of thaw.

4.4.2.20.1. If more than four hours elapse between thawing and infusion, an aliquot of the product should be removed immediately before administration to the patient to determine the cell viability of the infused product.

4.4.2.21. Remove a small volume from the reconstituted product for Complete Blood Counts (CBC), CFU, CD34+ counts and viability and sterility samples (bacterial and fungal cultures) as per transplant center procedures.

You may now call the Transplant Unit and advise them that the product is ready for infusion - if you do not intend to remove the cryoprotectant (step 4.5).

4.5. **Removing (washing) the cryoprotectant**

4.5.1. Place the transplant bag and the transfer bag in a centrifuge cup.

4.5.2. Fully support the transplant bag with inserts to prevent formation of creases during centrifugation as shown in the diagram below (Figure 9). Make sure SC-1 is closed.

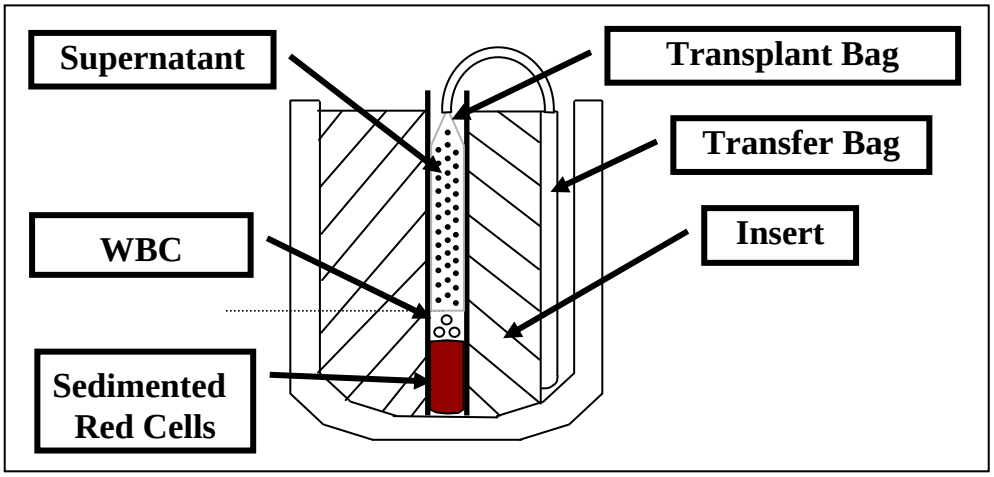

Figure 9.

4.5.3. Centrifuge at 400 x G, for 20 minutes, at 10°C.

You may now call the Transplant Unit and advise them that the transplant will be ready in about 30 minutes!

4.5.4. After centrifugation, remove with care, without disturbing the cellular pellet, the transplant bag from the centrifuge bucket.

4.5.5. Place the transplant bag in the plasma extractor.

4.5.6. Use SC-1 to adjust the flow and very slowly transfer approximately 2/3 of the supernatant (Supernatant-1) to the transfer bag avoiding the passage of cells.

Leave approximately 1/3 of supernatant with the cells (sedimented white and red cells in the diagram above).

NOTE: If you detect passage of cells to the transfer bag, return the contents to the transplant bag, resuspend the cells and repeat the centrifugation or centrifuge only the Supernatant-1 bag as described below in step 4.5.14.

4.5.7. Empty the tubing between the bags by pushing air from the transfer bag to the transplant bag. Close SC-1.

4.5.8. Seal the tubing between the bags close to the transplant bag. Cut through the seal and disconnect the transfer bag with the Supernatant-1 from the transplant bag with the cellular pellet (product).

4.5.9. Resuspend the cellular pellet by slowly adding with a syringe 25-50 mL of the dextran-albumin solution through the IP-1, with continuous mixing. The resuspended cells constitute the Sediment-1 (the graft).

4.5.10. The weight of the empty transplant bag if cut and sealed as shown below (Figure 10) is 23.6 g.

4.5.10.1. Calculate the weight of the Sediment-1 by weighing the filled transplant bag and subtracting 23.6 g.

4.5.11. Remove a small volume from the Sediment-1 for cell count, viability determination and sterility (bacterial and fungal cultures).

4.5.12. The recommended expiration time for HPC-C product **after the removal of the cryoprotectant** is 24 hours from the date and time of thaw. Store the product at 4˚C in a blood storage refrigerator until the product is issued.

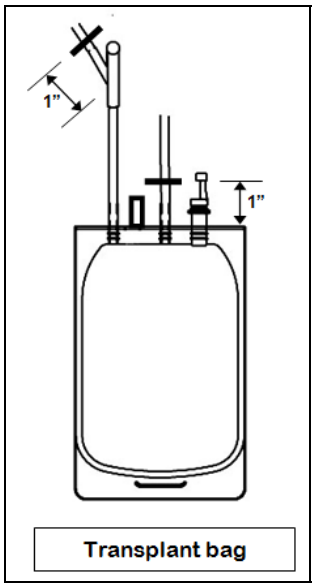

Figure 10.

4.5.13. <u>Inspect the supernatant for escaped cells</u>, even if there is no appearance of escape.

4.5.13.1. Express 10 mL from the Supernatant-1 bag into a conical centrifuge tube (accurate volume will help the accuracy of estimations).

4.5.13.2. Centrifuge at 600 x G, for 10 minutes.

4.5.13.3. Carefully aspirate 9.5 mL of supernatant without disturbing the (possible) cell pellet in the tip of the tube.

4.5.13.4. Resuspend the cell pellet thoroughly in the 0.5 mL of supernatant and load into a cell-counting chamber.

4.5.13.5. Count the nucleated cells per μL and calculate the total number of cells in the remaining volume of Supernatant-1.

4.5.13.6. Determine the number of nucleated cells in Supernatant-1 per Kg of patient's weight.

NOTE: A decision can then be made by the Transplant Physician on whether these cells should be added to Sediment-1 cells (the graft). This may not be necessary when the graft, Sediment-1, already contains a high cell dose. If the Sediment-1 cell dose is low, or borderline, a meaningful addition to the cell dose may be useful and desirable. If so, proceed to 4.5.14.

4.5.14. If escaped cells are to be collected from the Supernatant-1-containing bag, centrifuge the bag at 400 X G for 20 minutes at 10˚C to sediment the cells.

4.5.14.1. After centrifugation, connect a 300 mL transfer bag to the bag with Supernatant-1 in a laminar flow hood.

4.5.14.2. Position the bag in the plasma extractor and express the new supernatant (Supernatant-2) into the transfer bag so as to leave the sedimented cells (Sediment-2) in the original transfer bag.

4.5.14.3. Seal the tubing between the bags, cut through the seal and disconnect the transfer bag with the Supernatant-2 from the bag with the Sediment-2.

4.5.14.4. Resuspend the Sediment-2 in 10-15 mL dextran 40-albumin solution using a syringe and mixing gently.

4.5.14.5. Weigh the Supernatant-2 bag and the Sediment-2 bag and calculate the volumes by subtracting the weight of the empty bags similarly sealed.

4.5.14.6. Remove a small volume from the Sediment-2 for cell count, viability determination and sterility testing.

NOTE: **The volumes injected in step 4.5.9. may be modified if the Transplant Physician prefers injecting the patient with a smaller or larger total volume. In this case, resuspend the cellular pellet to the desired volume by injecting the corresponding volume of dextran-albumin solution.**

4.5.15. Bring the transplant bag (Sediment-1 bag) to the Transplant Unit even if the second bag (Sediment-2 bag) is being prepared; the second bag can be infused separately afterwards.

5. ADMINISTRATIVE REQUIREMENTS

5.1. Prepare a report on the procedure. Note the condition of the bag and whether and at what stage leaks or cracks were detected.

5.1.1. Include in the report:

5.1.1.1. Unit number

5.1.1.2. Date of receipt of the Unit

5.1.1.3. Liquid Nitrogen Storage conditions in your facility

5.1.1.4. Date of thawing

5.1.1.5. Volume of the final product

5.1.1.6. Total nucleated cell (TNC) count, CD34+ content

5.1.1.7. Viability of the cells recovered (TNC or CD34+ cells) and the method used

5.1.1.8. Results of bacterial and fungal cultures.

5.2. E-mail or fax a copy of the report to the cord blood bank (NCBP) from which you have received this unit (ncbp@nybloodcenter.org). Fax: (718)-707-3747.

5.3. Keep a copy for your Processing lab records.

5.4. Return the dry shipper to the New York Blood Center – National Cord Blood Program.

The return address is:

New York Blood Center
National Cord Blood Program
45-01 Vernon Blvd.
Long Island City, NY 11101
Ph: (718)-706-5211
Fax: (718)-707-3741

6. *EMERGENCY PRODUCT RECOVERY IN THE EVENT OF A CONTAINER FAILURE*

6.1. HPC-C product bags are very fragile.

6.1.1. To prevent accidental fracture, the bags must be handled with extreme caution when removing them from the protective metal cassettes, during inspection and during the thawing process.

6.1.2. It is recommended that the thawing process to be performed in a controlled laboratory environment which provides appropriate equipment and supplies for post-thaw sampling and/or bag rescue, as well as dedicated space and personnel for product preparation.

6.1.3. To mitigate the extreme temperature change from storage at -196˚C (Liquid Nitrogen phase) to thawing at 37˚C and possible sudden vaporization of liquid nitrogen in recessed of the bag or tubing, transfer the bag with the product from the liquid phase of nitrogen to the vapor phase for a period of time (a few minutes) before removal for thawing.

6.1.4. To prevent an accidental drop onto the floor, HPC-C bags must be handled over a flat surface, such as a table.

6.1.5. It is recommended that all HPC-C products be placed in individual sterile zipper-locked bags prior to thawing to facilitate salvage of the product and reduce contamination in case of an unanticipated problem.

6.2. If the HPC-C bag is obviously fractured upon removal from cold storage, or if it fractures during the thawing process, please notify the NYBC NCBP Processing Laboratory as soon as possible (phone number: 718-706-5211).

6.2.1. Notify the transplant physician and the laboratory director immediately.

6.2.2. It is the transplant physician's (or designee's) responsibility to determine whether the HPC-C product will be used or discarded and whether additional HPC-C product(s) are to be requested for infusion.

6.2.3. If the transplant physician (or designee) determines that the product in the ruptured bag should be used, the HPC-C product may be recovered as follows:

6.2.3.1. Place the ruptured bag into the sterile zipper-locked plastic bag to prevent further loss and/or contamination of the product during the thawing process.

6.2.3.2. Thaw the product according to the transplant center's standard procedure.

6.2.3.3. Small leaks or tears of the ruptured bag can be blocked off with hemostat clips.

6.2.3.4. Withdraw the thawed product from the freezing bag and any product from the zipper-locked bag into one or more 60 mL syringe(s) with sterile tubing attached.

6.2.3.5. The product may be transferred via sterile syringe inside a biological safety cabinet into a new container. This new bag could be either the sterile Transplant bag that is provided with the product or a bag of a stocked salvage kit that should be readily available in the thawing laboratory for use in these situations.

6.2.3.6. Save an aliquot of the product and send for gram stain and bacterial and fungal cultures.

6.2.3.7. Dilute (reconstitute) the thawed product and remove the cryoprotectant according to the procedure described above or administer the diluted product to the patient as per transplant physician's instructions.

6.2.3.8. It would be the transplant physician's (or designee's) responsibility to determine whether to treat the patient with broad-spectrum antibiotic coverage and the necessity for an infectious disease consultation.

6.2.3.9. If possible, please save the ruptured bag (with or without HPC-C) and place it into a biohazard bag.

6.2.3.9.1. Notify NCBP staff. NCBP staff will notify the manufacturer and provide information for returning the bag to the manufacturer for evaluation.

7. REFERENCES

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7.4. FDA Guidance for Industry: Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic Reconstitution for Specified Indications. October 2009.

Summary of Serious Post-Infusion Adverse Reactions of NCBP CB Units

#	NCBP Search ID	Patient CIBMTR ID	NCBP Unit ID	Tranplant date	Type of transplant (single/double CBU) Other manipulation	Event Date	Event description	Initial Report Date	Reported by	CBU processing date / method	Review of NCBP records - Findings
1	**4938**	NA	[redacted]	**09/25/1997**	single	**09/25/1997**	pt received his autologous (unknown at the time) CBU	06/12/2008	NCBP	3/20/1997 - 2	review of collection site records identified name
2	**6893**	NA	[redacted]	**12/10/1998**	single	**12/10/1998**	wrong CBU shipped and infused		TC MD	8/17/1993 - 1	incorrect CBU identification
3	**17721**	NA	[redacted]	**09/01/2005**	double (CBU [redacted] expanded)	**09/01/2005**	SOB, pericardial effusion, tamponade intubated, died [redacted] >48h post infusion	submitted with Tx Report	TC DM	1/20/2000 - 3	no unusual findings
4	**25684**	[redacted]	[redacted]	**04/14/2009**	double	**04/14/2009**	hypertension, chest pain, desaturation	04/23/2009	NMDP	12/29/2000 - 3	no unusual findings
					CBU [redacted] was second first CBU was RBC replete		decrease in cardiac function, intubation				
5	**24605**	[redacted]	[redacted]	**01/29/2009**	double CBU [redacted] was first unit Infusion aborted	**01/29/2009**	severe SOB, tightness, chest/abd pain sweating, desaturation, tachycardia medical treatment, no intubation	01/29/2009	TC physician	4/18/2006 - 3	no unusual findings
					Two other CBU infused						
6	**29009**	[redacted]	[redacted]	**09/22/2010**	double	**09/23/2010**	evelation of serum creatinine acute renal failure	10/01/2010	NMDP	5/1/2002 - 3 1/5/2004 - 3	no unusual findings post-processing Hcts 70.4% and 47.3%

Summary of Serious Post-Infusion Adverse Reactions of NCBP CB Units

#	NCBP Search ID	Patient CIBMTR ID	NCBP Unit ID	Review of TC records - Findings	Final Report	Follow-up	Comments/Corrective Action
1	**4938**	NA	[redacted]	none	pt died on [redacted]	pt died	change in release procedure
					multiorgan failure		for 6/6M units
							to be included in FDA Annual Report
2	**6893**	NA	[redacted]	post-transplant HLA typing with	pt received wrong CBU MM at	pt had BM tx	change in release procedure
				"unknown" antigen	3/6 loci instead of 5/6		HR DRB1 typing at CT
3	**17721**	NA	[redacted]	Discharge - Death summary	cyclophosphamide induced	patient died	Included in FDA Annual Report
					cardiomyopathy		
4	**25684**	[redacted]	[redacted]		cardiac toxicity	recovered	NMDP BB-IND#7555
						engrafted	Included in Report to FDA 8/31/09
5	**24605**	[redacted]	[redacted]	successful infusion of two other	anaphylactic reaction	recovered	similar reaction later to platelets
				thaw/washed CBU (one AXP)	?idiosyncratic	engrafted	to be inlcuded in FDA Annual Report
				similar reaction later to platelet		discharged	
				trasfusion			
6	**29009**	[redacted]	[redacted]	albumin reconstitution post thaw	acute renal failure	recovered	to be inlcuded in FDA Annual Report
			[redacted]	TV: 100 ml each unit		engrafted	
				hypertension during infusion		discharged	

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