Public Cord Blood Banking and unrelated Transplantation
National Cord Blood Program

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Cord Blood in hematopoietic stem cell transplantation

- The blood remaining in the placenta and umbilical cord after the birth of baby is rich in hematopoietic stem cells.
- This blood can be harvested easily, with no risk to the mother or baby.

**Advantages of CB in transplantation**

- Easy access – large numbers of donors – ethnic diversity
- Can be collected and stored (cryopreserved) in CB Banks
- Available upon demand – no delays in transplants
- Lower risk of viral infections
- Immunologically “naïve” T cells: do not need “perfect” match

**Considerations**

- Cell dose (volume)
- Transmission of (unknown) diseases
Public CB Banking at the National Cord Blood Program (NCBP)
Cord Blood Collection

- Ex utero collection
- Trained collection personnel
- TNC count at collection - Identification of clinical units
- Maternal Consent - sample - Questionnaire - Records
- Transportation to the Bank - temperature monitoring

Time from collection to completion of processing: maximum 36 hours
What is a “bankable” CBU

- “Adequate” TNC (volume): above Bank’s “cut-off”
- Not Clotted
- Maternal informed consent – medical information
- No Contamination with Bacteria/Fungi
- No Contamination with Mother’s Blood
- Processed within 36 hours* from collection
- Adequate / Viable Hematopoietic Progenitor Cells
- Complete infectious disease testing

*FACT requires maximum of 48 hrs from collection
The “life cycle” of a CBU

Permission to collect - review medical record

Harvest CB

TNC

Total Nucleated Cell count at collection

Label CBU - Clinical - Informed consent – maternal questionnaire

Processing - Cryopreservation - Freezing

Testing: Potency, IDMs, eligibility, HLA, other

QA Review: CBU Release to Search (status)

Review for patient: TNC/match HLA CT from segment

CBU Release for Transplant

Research CBU
No identifiers
No further tests
Cord Blood Processing

AXP: Automated Processing System

- Partial RBC depletion and volume reduction
- Closed manufacturing system; Aseptic processing
- Accurate final product volume; Consistent, low hematocrit
- High recovery of mononuclear and CD34+ cells; excellent viability
Cord Blood freezing and storage

Two-compartment Cryopreservation Bag; total volume: 25 mL
HPC-C cryoprotected in DMSO; final DMSO concentration: 10%

Segments: identity and potency testing post-cryopreservation
1: HLA Confirmatory Typing; 2: CD34+ count/viability, CFU testing
3: retention sample (FDA)
Cord Blood cryopreservation and storage

BioArchive freezer

Individual CBU:
- controlled rate freezing (CRF)
- long-term storage in liquid nitrogen in the same freezer
- reduced transient warming events
- automated retrieval

Standardization:
- highly reproducible cryopreservation profiles
- computerized system

Capacity: 3600 HPC-C products
Cord Blood Testing

- Maternal blood sample: donor ID screening including NAT for HIV/HCV/HBV and WNV, and testing for CMV
- CB: CBC, CD45+/CD34+ cells and viability, CFU assays
- CB: ABO, Rh, SS hemoglobin (HPLC); molecular Hb testing as needed
- CB: Bacteriology (bacterial, fungal, aerobic, anaerobic)
- CB: Testing for relevant genetic diseases can be performed prior to transplant
- HLA typing (class I, II performed at the DNA level)
Evaluation of HPC-C Potency by a standardized high throughput CFU strategy using High-Resolution Digital Imaging

Unstained image

Stained image

Documentation
Testing date/time
CBU ID - sample
Dish ID
Operator
Hood

Advantages
Standardized assay
High numbers (30 CBUs/day)
Stain: enhances detection
Stored digital images

○ = CFU-GM
○○ = BFU-E
□ = CFU-mixed
Standardization of CFU potency assay

CFU ASSAY: LIMS and HRDI-ACC APPROACH
Potency: CFU counts and vCD34+ cells

N=11,587 CBU, post-processing samples

\[ R^2 = 0.76 \]
\[ Y = 1.4x + 0.86 \]
Cord Blood Shipping - Transportation

Shipping container

- CryoShipper Lid
- CB Unit
- < -150°C for 5-7 days

Continuous temperature monitoring during transportation

- MVE Data logger

-190°C
Access to the NCBP CB Inventory

NCBP’s Web portal: WebSearch

Registries:

• Single Point of Access – NMDP
• Bone Marrow Donors Worldwide
NCBP HPC, Cord Blood: final product

Highly regulated stem cell source
Accreditations: FACT or AABB
FDA Regulations (Public CB Banks)
HEMACORD November 2011
Stability studies of CB products:

a) Does the “time in storage” affect potency or engraftment ability

b) Is there an expiration date for the products?
# Stability Evaluation of CB products

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
<th>Stability-Indicating</th>
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</thead>
<tbody>
<tr>
<td>Visual Inspection of product after thawing</td>
<td>Determine Integrity of container and closures and Identity Label</td>
<td>Integrity, Identity</td>
</tr>
<tr>
<td>Total nucleated cell (TNC) count</td>
<td>Measuring TNC content</td>
<td>Potency</td>
</tr>
<tr>
<td>Viable CD34+ cell content (CBU segment and Bag)</td>
<td>Measuring CD34+ cell number and viability</td>
<td>Potency</td>
</tr>
<tr>
<td>Colony-Forming Units (CFU) (CBU segment and Bag)</td>
<td>Counting colonies of functional progenitor cells</td>
<td>Potency</td>
</tr>
<tr>
<td>Microbiology</td>
<td>Detection of microbial contamination</td>
<td>Integrity, Purity, Safety</td>
</tr>
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</table>

**Clinical CBU from all 4 NCBP manufacturing periods are used**

**Stability studies: CB Product Expiration Date**

Each Year, Expiration Date Extends
Manufacturing

PRODUCT NAME

Example: Labeling/Expiration date

NEW CONTAINER LABEL
APPLIED AT SHIPMENT

EXPIRATION DATE

“PARTIAL” LABEL
PRINTED AT SHIPMENT

HEMATOPOIETIC PROGENITOR CELLS, CORD BLOOD
HEMACORD
Injectable Suspension

HEMACORD ID: 123456P
RECIPIENT: Last, First
SEARCH ID: 11111
TNC/kg: 2.3 x 10^7

HLA match with recipient: one B locus mismatch
(One B locus mismatch is assigned considering low-resolution typing for HLA class I and B loci, and high-resolution typing for HLA DRB1 alleles.)

For Intravenous Administration Only
Do Not Irradiate
Rx only

Cryopreservation (concentration): DMSO (10%) / Dextran 40 (1%)
Volume: Approx. 25 mL
Storage: ≤ -150°C
CBU segment analysis for evaluation of CBU quality/potency post-cryopreservation

AXP CBU Attached Segments
1: HLA Confirmatory Typing
2: CD34+ count/viability;
   CD45+ count/viability;
   CFU testing
3: retention sample
NCBP CBU: segment CD34+ cell viability
N=1924 segments; N=1494 AXP CBU

CBU Processing method: AXP (automated)
Freezing - Storage: BioArchive freezers
Manufacturing Period: 2006 - 2016
Testing prior to CBU release for transplant

Scaradavou et al, ASH 2016
Segment CD34+ and CD45+ cell viabilities
NCBP CBU: segment CD34+ cell viability

N=684 segments; N=673 CBU (non-AXP)

mean 94.2
median 95.4
SD 4.3

Storage % CD34+ cell viability

CBU Processing method: manual
Freezing - Storage: BioArchive freezers (since 1999)
Testing at time of CBU HLA CT (recent)

Scaradavou et al, ASH 2016

Storage Time (yrs)
mean 10.6
median 10.0
min 6.3
max 21.0
CB graft-related variables that affect engraftment, survival and relapse
Unrelated Donor Searches: Results based on patient Ancestry

CB extends transplant access to patients of ethnic “minorities”

Data from Memorial Sloan-Kettering Cancer Center

Barker et al, BBMT 2010; 16(11):1541-1548
Time to engraftment

TNC/kg is the most significant variable affecting engraftment

Eligible for analysis: N=562; single unit grafts; Diagnoses: all; Transplant Centers: all (domestic/international); Tx period: 1992-1998

Rubinstein et al, NEJM 1998; 339:1565-77
Eligible for analysis: N=1202; single unit grafts; Diagnoses: all; Transplant Centers: domestic; Tx period: 1993-2006

*Stevens et al, Blood 2011; 118:3969-78*
Time to engraftment

Neutrophil Engraftment: Effect of TNC and HLA

Cox Regression

<table>
<thead>
<tr>
<th>CI of Neutrophil Engraftment</th>
<th>Days Post-Transplant</th>
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<tbody>
<tr>
<td>0 MM: all TNC, (mean 4.4)</td>
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<tr>
<td>1-2 MM, TNC ≥10.0</td>
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<tr>
<td>1-2 MM TNC 5.0-9.9</td>
<td></td>
</tr>
<tr>
<td>1-2 MM TNC 2.5-4.9</td>
<td></td>
</tr>
<tr>
<td>3 MM: all TNC (mean 3.7)</td>
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<tr>
<td>1-2 MM, TNC &lt; 2.5</td>
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Higher TNC may improve engraftment of HLA MM units

Eligible for analysis: N=1061; single unit grafts; Diagnoses: hematologic malignancies; Transplant Centers: all (domestic/international); Tx period: 1993-2006

Barker et al, Blood 2010; 115: 1843-1849
Post-thaw CD34+ cell viability as indicator of CBU potency

Post-thaw CD34+, CD3+ and CD45+ cell viability and unit engraftment in 44 double unit CB grafts

Modified ISHAGE gating strategy for assessment of post-thaw cell viability

Scaradavou et al, BBMT 2010; 16: 500-508
Time to ANC engraftment by infused viable CD34+ cell dose

Infused viable CD34+ cell dose: critical determinant of neutrophil engraftment

Purtill et al, Blood 2014; 124: 2905-12
Number of HLA MM and post-transplant events

0 MM grafts appear to have the best outcomes

Eligible for analysis: N=562; single unit grafts; Diagnoses: all; Transplant Centers: all (domestic/international); Tx period: 1992-1998

Rubinstein et al, NEJM 1998; 339:1565-77
CIBMTR study: effect of allele level match on Non-Relapse Mortality and Overall Survival after single unit CB transplants

Eligible for analysis: N=1568; single unit grafts; Diagnoses: hematologic malignancies; Conditioning: myeloablative; Transplant Centers: all; Tx period: 2000-2010

Eapen et al, Blood 2014; 123: 133-140
The **IPA/NIMA** effects during pregnancy

### NIMA
- non inherited maternal antigens

### NIPA
- non inherited paternal antigens

### IMA
- inherited maternal antigens

### IPA
- inherited paternal antigens

**Transplacental trafficking:**

The fetus gets exposed and develops immunity and T regulatory cells against the NIMA.

**Maternal microchimerism:**

The mother gets exposed, and develops B and T cell immunity against the IPA of the fetus.

Exposure to NIMA/IPA has implications when CB is used for transplant
CB unit selection for transplant: Steps

1. **Search for domestic and international CBU**

2. **“Screen” CBU by TNC**: Establish a TNC dose “threshold” depending on graft (single / combined / other sources / expansion)
   - **minimum**: 2-3 x10^7/kg for single CBU
     - 1.5-2 x10^7/kg for each of the CBU in a double graft

3. **For CBU above the “threshold” TNC dose:**
   - **evaluate HLA match level** (at 6 and 8 alleles preferably):
     - If fully matched CBU: best choice (CBU quality needs to be considered)
     - avoid CBU with < 3/8 allele match, if possible
     - consider “permissible” mismatches for hematologic malignancies (unidirectional HLA MM, maternal HLA phenotype for NIMA/IPA assignments)
     - do not limit selection based on unit-unit match
   - **evaluate potency assays**, if available; presence of CBU segment
   - **evaluate CB Bank of origin**; overall quality of products
   - **consider other patient-related variables** (DSA, RBCs, CBU volume)

4. **Identify CBU for the graft and back-up**
National Cord Blood Program

- Collections - Rodica Ciubotariu, MD, PhD
- Quality Control - Susana Albano, PhD
- IT Systems - Michal Tarnawski, MD
- Manufacturing - Ludy Dobrila, PhD
- Validations – Connie Cheung
- Medical Director - Andromachi Scaradavou, MD
- Program Director - Pablo Rubinstein, MD

- QRA: Betsy Jett, VP QRA
  - NYBC QRA staff assigned to NCBP