Share the Science

Quality Cord in Cord Blood Banking

Presented by:

Susana Gomez, Ph.D.
Head of Cord Blood Bank
Anthony Nolan Cell Therapy Centre
Share the Science Webinar Series

- Educational Partnership
- Focus on industry topics and trends
  - Research
  - Expert opinions
  - Industry news
- Expert speakers
Introducing Mediware

- **History**
  - Incorporated in 1980
  - Over 3,000 installed customer facilities
  - Over 400 employees and now a private company backed by Thoma Bravo private equity

- **Products and Solutions**
  - Blood & Cellular Therapy Management
  - Medication Management
  - Performance Management
  - Home Care (a.k.a. “Alternate Care”)

- **Installations across the world including**
  - U.S., Canada, Ireland, U.K., South Africa, Holland, Belgium and Singapore

- **Locations - Headquarters: Kansas City**
  - Chicago, Jacksonville, Atlanta, Providence (RI), Pittsburgh, Indianapolis, St. Louis, Chandler, AZ
  - Merged with Thoma Bravo in 2012 for the purpose of going private – previously listed on NASDAQ
Transtem Cellular Therapy Software

- Two Primary Component Offerings:
  - **Transtem Lab**: For Cellular Therapy Labs, Cord Blood Banks and Regenerative Medicine Programs
  - **Transtem Patient**: For Cancer Centers and Bone Marrow Transplant (BMT) Programs

- Bridges donor, product and patient information into a single environment
- Improves how we access and share information
- Drives compliance with standards and regulatory bodies
- Provides comprehensive management of stem cell transplants
- Provides strong analytical tools for program quality improvement and to drive research
- Allows for the management of multiple business lines through a single system
Introducing Save the Cord Foundation

For more information about Save the Cord Foundation and saving cord blood, please visit www.savethecordfoundation.org.
Introducing Dr. Susana Gomez

Susana Gomez, Ph.D.

Head of Cord Blood Bank
Anthony Nolan Cell Therapy Centre (ANCTC), Nottingham
QUALITY IN CORD BLOOD BANKING

Dr Susana G Gomez
15th October 2015
CORD BLOOD BANK STANDARDS

The major objective of the NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration is to promote quality medical practices, laboratory processes, and banking to achieve consistent production of high quality placental and umbilical cord blood units for administration. These Standards apply to cord blood units intended for unrelated allogeneic use and to related units collected and stored for allogeneic or autologous use by donor family. All phases of cord blood collection, banking, and release for administration are included:
A survey was sent out to all WMDA members to evaluate the parameters considered to be most important when selecting cord blood units for transplantation.

The outcome of the survey would help us to evaluate the effectiveness of our processing techniques and highlight areas for improvement.

It would also identify the Key performance indicators (KPI’s) as dictated by cord blood users.

The Survey should also identify any gaps between the what Cord blood providers strive for and what Cord blood users want at this current time.
The Survey was sent out in the first quarter of 2013. Of all the WMDA members the survey was sent to, 50 responded. The breakdown of the respondents by background are detailed below.

The remaining analysis will be broken down into two groups, The **providers** (cord blood processing background) and the **selectors** (Registry and transplant background).
The Survey asked for the respondents to rate the following parameters out of 10 (1 being not at all important 10 being extremely important)

- Pre Freeze Total Nucleated Cells (TNC)
- Pre Freeze Mononuclear Cell (MNC)
- Pre Freeze CD34+
- Pre Freeze % Granulocyte
- Pre Freeze CD45+ viability
- Pre Freeze CD34+ viability
- Pre Freeze Haemotocrit
- Pre Freeze potency (CFU)
- Post thaw Nucleated Cell (NC) yield
- Post thaw CD34+ yield
- Post thaw CD45+ viability
- Post thaw CD34+ viability
- Post thaw potency (CFU)
The Score for each parameter is given below subdivided by group

- Pre Freeze % Granulocyte
- Pre Freeze Mononuclear Cell (MNC)
- Pre Freeze Haemotocrit
- Pre Freeze potency (CFU)
- Pre Freeze CD45+ viability
- Post thaw CD45+ viability
- Pre Freeze CD34+ viability
- Post thaw potency (CFU)
- Pre Freeze CD34+
- Post thaw CD34+ yield
- Post that CD34+ viability
- Post thaw Nucleated Cell (NC) yeild
- Pre Freeze Total Nucleated Cells (TNC)
Reviewing this information, there are a few important things to note:

**Nothing scored lower than 4** by the total group. Although individuals scored lower, averagely the group or subgroup didn’t consider any parameters to be of no importance suggesting that more information at selection is better.

There was largely uniformity across the group with no significant difference observed between the subgroups with the exceptions of **post thaw nucleated cell yield** which the cord blood selectors considered to be more important than the cord blood providers did.

The **Cord blood selectors group appears more uniform** with there answers showing considerably lower Standard deviations compared to the cord blood providers group. However this could also be an artefact of the smaller sample size.
Below is a list of the top 4 scoring parameters for each group

<table>
<thead>
<tr>
<th>CORD BLOOD PROVIDERS</th>
<th>CORD BLOOD SELECTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post thaw CD34+ viability</td>
<td>Pre Freeze Total Nucleated Cells (TNC)</td>
</tr>
<tr>
<td>Pre Freeze CD34+</td>
<td>Post thaw Nucleated Cell (NC) yield</td>
</tr>
<tr>
<td>Pre Freeze Total Nucleated Cells (TNC)</td>
<td>Post thaw CD34+ viability</td>
</tr>
<tr>
<td>Post thaw CD34+ yield</td>
<td>Post thaw CD34+ yield</td>
</tr>
</tbody>
</table>

If we considered this like an algorithm we might expect to see:

**Cord Blood Provider**
- Stem Cell durability
- Total Stem cells (accurate enumeration)
- Total TNC

**Cord Blood Selector**
- Total TNC
- Stem Cell durability
Although on the whole the entire group seems to be in agreement regarding the most important parameters when selecting cords and therefore for processing cords, as we start to tease apart the data we start to see differences. This serves as an example of how the two groups view cord.

**Cord Blood Providers**: Evaluate based on specific and accurate testing methodologies

**Cord Blood Selectors**: Evaluate based on easily standardised testing methods with proven cross centre reproducibility and parameters with long standing correlations to engraftment.
Cord Blood selectors and users desire a much higher TNC to be available at point of transplant. (note it would be good to find out the average transplanted to discern between desired the actual)

Interestingly, Cord blood providers put a higher requirement on the total CD34+ Cells

saving the lives of people with blood cancer
CONCLUSION:

• It is Clear that **units are selected according to TNC** and that Cord blood users desire units that are much higher than thresholds currently set by many cord blood inventories.

• There needs to be a mutual understanding of cord blood parameters and there limitations

• There needs to be communication between these groups to allow for the Cord Blood Providers parameters to be translated to standardised testing methods with proven cross centre reproducibility and parameters with long standing correlations to engraftment.
Table 2  Required and recommended tests and test results according the US FDA for cord blood and HPC-C (final cord blood product)\(^a\)

<table>
<thead>
<tr>
<th>Product characteristics</th>
<th>Testing</th>
<th>Sample (type and timing)</th>
<th>Results of product testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td>Infectious diseases—testing required (21 CFR 1271.45 through 1271.90)</td>
<td>Maternal peripheral blood obtained within 7 days of cord blood collection—type and timing required. (21 CFR 1271.80(a) and (b))</td>
<td>All tests negative except non-treponemal test for syphilis when confirmatory test is negative. CMV results are recorded. CMV report</td>
</tr>
<tr>
<td></td>
<td>Sterility—bacterial and fungal cultures—testing required. (21 CFR 211.165(b) and 21 CFR 610.12)</td>
<td>HPC-C (^a)(pre-cryopreservation)</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Haemoglobin</td>
<td>Cord blood(^b) or appropriate donor sample obtained at time of cord blood recovery</td>
<td>No homozygous haemoglobinopathy</td>
</tr>
<tr>
<td>Purity and potency</td>
<td>Total nucleated cells (TNCs)</td>
<td>HPC-C (pre-cryopreservation)</td>
<td>(\geq 5.0 \times 10^8) TNC(^c) per unit HPC-C</td>
</tr>
<tr>
<td></td>
<td>Viable nucleated cells</td>
<td>HPC-C (pre-cryopreservation)</td>
<td>(\geq 85%) viable nucleated cells</td>
</tr>
<tr>
<td></td>
<td>Viable CD34(^+) cells (flow cytometry)</td>
<td>HPC-C (pre-cryopreservation)</td>
<td>(\geq 1.25 \times 10^9) viable CD34(^+) cells(^d) per unit</td>
</tr>
<tr>
<td>Identity</td>
<td>HLA typing</td>
<td>HPC-C (pre-cryopreservation)</td>
<td>HPC-C</td>
</tr>
<tr>
<td></td>
<td>Confirmatory HLA typing</td>
<td>Attached segment of HPC-C</td>
<td>Report</td>
</tr>
<tr>
<td></td>
<td>Blood group and Rh type</td>
<td>Cord blood</td>
<td>Confirms initial typing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Report</td>
</tr>
</tbody>
</table>

Abbreviation: FDA = Food and Drug Administration.

\(^a\)Sample may be obtained before or after addition of the cryoprotectant.

\(^b\)Cord blood = cord blood before undergoing volume reduction.

\(^c\)Based on 20kg recipient, a target dose of \(\geq 2.5 \times 10^7\) nucleated cells per kg and 70\% post-thaw recovery = \(1.7 \times 10^7\) nucleated cells per kg.

\(^d\)Based on CD34\(^+\) cells \(\geq 0.25\%\) of TNC before freezing.
COLLECTION
• High cell content

FRESH TRANSPORTATION
• High viability
• Traceability

PROCESSING
• High TNC, MNC, CD34 yields
• High viability

STORAGE
• Long term storage
• TWE

SHIPMENT
• Cryogenics temperatures
• Traceability

saving the lives of people with blood cancer
QUALITY CRITERIA (SIPP)

• **SAFETY**
  – Sterility
  – IDM / Virology
  – Haemoglobinopathies

• **IDENTITY**
  – HLA Tissue Type – High Resolution
  – Blood Group

• **PURITY**
  – TNC
  – CD34

• **POTENCY**
  – Viability
  – Colony Forming Unit CFU assay
SAFETY

IDM/Virology

Table 2. Testing requirements when umbilical cord blood cells are stored for allogeneic use:

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Serology Testing after 180 days</th>
<th>Alternative Analytical Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>Anti-HIV-1,2</td>
<td>HIV-NAT</td>
</tr>
<tr>
<td>HBV</td>
<td>HBsAg and anti-HBc</td>
<td>HBV-NAT</td>
</tr>
<tr>
<td>HCV</td>
<td>Anti-HCV-Ab</td>
<td>HCV-NAT</td>
</tr>
<tr>
<td><em>T. pallidum</em> (syphilis)</td>
<td>Anti-<em>T. pallidum</em></td>
<td>Not required</td>
</tr>
</tbody>
</table>

- HTLV I-II, HTLV NAT
- CMV (IgG, IgM, PCR-as confirmatory)
- EBV (IgG, IgM),
- Toxoplasmosis (IgG, IgM),
- Malaria & Chagas (when required)
SAFETY

Sterility / BACTEC®
**IDENTITY**

**HLA tissue typing & blood group**

<table>
<thead>
<tr>
<th>AN cord No.</th>
<th>GB2931358</th>
<th>Local Cord Ref.</th>
<th>ANCBB 116896 C</th>
<th>Weight (kg)</th>
<th>N/A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age.</td>
<td>N/A.</td>
<td>Sample Bled.</td>
<td>N/A.</td>
<td>Pregnancies</td>
<td>N/A.</td>
</tr>
<tr>
<td>Sex Determination</td>
<td>Female</td>
<td>Sample Received:</td>
<td>08/05/2015</td>
<td>Lab Sample No:</td>
<td>10773235 / 10800911</td>
</tr>
<tr>
<td>External Cord Ref.</td>
<td>G221213116896</td>
<td>Sample Type:</td>
<td>Cord Segment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HLA Typing Results:**

<table>
<thead>
<tr>
<th>HLA-A</th>
<th>*01:01/01L.</th>
<th>*03:01:01.</th>
<th>2,4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLASS I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-B</td>
<td>*39:01/01L.</td>
<td>*18:01:01.</td>
<td>2,4</td>
</tr>
<tr>
<td>HLA-C</td>
<td>*07:01.</td>
<td>*12:03.</td>
<td>2,4</td>
</tr>
<tr>
<td><strong>CLASS II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*01:01/50.</td>
<td>*04:01:01.</td>
<td>2,4</td>
</tr>
<tr>
<td>HLA-DRB3</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HLA-DRB4</td>
<td>*01:01/03/06/08.</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HLA-DRB5</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HLA-DQA1</td>
<td>*01:01/04/05.</td>
<td>*03:01/02/03.</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DQB1</td>
<td>*05:01/12/18.</td>
<td>*03:02.</td>
<td>2</td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>Not Tested.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Date HLA Tests Completed: 15/05/2015

Date HLA Tests Completed: 19/09/2013
PURITY

TNC count

- Initial counts
- Final counts
The UCB protocol is designed for routine processing of umbilical cord blood (UCB) to concentrate the buffy-coat fraction. TNC recovery is achieved without addition of any sedimentation agent. The UCB protocol allows a volume reduction of UCB in approximately 35 min to a predetermined fixed volume ranging (21 ml). The input volume range is 35 to 290 ml.
PUiRITY

TNC count

- CESCA THERAPEUTICS-AXP®

- Automates the volume reduction process
- Provides consistent buffy coat concentration volumes
- Ensures high recoveries of mononuclear cells (MNCs) in a targeted volume
- Allows for simultaneously processing of multiple cord blood units in one centrifuge
PURITY

TNC count

- SYNGEN-SynGenX™-1000

- Advanced Technology for Cell Separation and Cryopreservation
- High CD34, MNC and TNC Recovery and Post Thaw Viability
- Critical Process Control with Electronic Records
- Easy to Use for Both Small and Large Scale Operations

saving the lives of people with blood cancer
PURITY

CD34 count
POTENCY

Viability (7AAD & Annexin V)

Dominant unit CD34+ cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice

Duncan Purtill,1 Katherine Smith,2 Sean Devlin,3 Richard Meagher,2 Joann Tonon,2 Marissa Lubin,1 Doris M. Ponce,1,4 Sergio Giralt,1,4 Nancy A. Kernan,5 Andromachi Scaradavou,5 Cladd E. Stevens,1 and Juliet N. Barker1,4

1Adult Bone Marrow Transplantation Service, Department of Medicine, 2Department of Laboratory Medicine, 3Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; 4Weill Cornell Medical College, New York, NY; and 5Bone Marrow Transplantation Service, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY

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A

Correlation r² = 0.73
Median recovery: 101%
< 75% viability: 8%
POTENCY

CFU assay

- 14 days assays—although there is a possibility for doing in 7 days
- Different companies who provide methylcellulose
- Manual reading—subjective to operator
COLLECTION
• High cell content

FRESH TRANSPORTATION
• High viability
• Traceability

PROCESSING
• High TNC, MNC, CD34 yields
• High viability

STORAGE
• Long term storage
• TWE

SHIPMENT
• Cryogenics temperatures
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saving the lives of people with blood cancer
Prof Alejandro Madrigal
Dr Sergio Querol
Dr Roger Horton
Mr Daniel Gibson
Mr Chris Leonforte
All lab scientist at CBB
All staff at collection sites

All our great mums and babies who have donated their cords!
Questions?
Contact:

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charis.ober@savethecordfoundation.org