Second generation of Cord Blood Banks

Dr Sergio Querol, Dra Marta Torrabadella, Dr Joan Garcia, Prof Alejandro Madrigal

Anthony Nolan Research Institute, London & Programa Sang de Cordó, Barcelona
Blood, 1974, Vol. 43, No. 3, pp. 357-361

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
BM versus UCB in Adults

Not so good for UCB—why?

1) low cell dose
2) 2 antigen HLA mismatched
3) learning curve
TOTAL NUMBER OF CORD BLOOD UNITS PROVIDED

UNRELATED CORD BLOOD BANKS/REGISTRIES 2007
Figure 5: Degree of matching of the cord blood units provided for children (N=1,206 units)*

- 0 mismatched: 219 units
- 1 mismatched: 535 units
- 2 mismatched: 362 units
- 3 or more: 11 units
- Not specified: 79 units
Figure 6: Degree of matching of the cord blood units provided for adults (N=1,420 units)*

*From 117 cord blood units no information is available whether the unit has been used for child or adult patient
Cord blood banks: The research phase

• Clinical success of first generation of cord blood banks:
  - The Eurocord/Netcord synergy
  • Clinical assessment and advice
  • Quality (International standards)
But, what is a Cord Blood Bank?

2001: Standards for Cord Blood Services

Guidance for Industry

Minimally Manipulated, Unrelated, Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic Reconstitution in Patients with Hematological Malignancies

DRAFT GUIDANCE

This guidance document is for comment purposes only.

Submit comments on this draft guidance by the date provided in the Federal Register notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.fda.gov/dockets/comments. You should identify all comments with the docket number listed in the notice of availability that publishes in the Federal Register.

Additional copies of this draft guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFMA-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/over/guidelines.htm.

For questions on the content of this guidance, contact Ellen Lazarus, M.D., at 301-827-6031.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologies Evaluation and Research
December 2006
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Threats

- **Efficiency:**
  - <30% collected, stored
  - <1% stored, transplanted

- **Financial:**
  - New limits, no break-even
  - Continuously increasing transplant costs
1000 units collected, €470,000

Collection/transport: ~€150

1 billion cells?

Discard

60%

€90,000

Clinical

Accredited process

Processing/Testing: ~€400

40%

€380,000

First generation of cord blood banks

Break-even (1%) = €47,000

- Public funding = National programmes
- Charitable funding
Questions

• Which size? Cost-benefit study

• How do we get this size promptly? Financial engineering

• How do we obtain the most of cord blood? Pharmaceutical approach
Opportunities

- Excellent biological profile
- Non-questioned ethical advantage
- Social awareness
- Individual demand
- Stem cell revolution: grants
- Research needs: partnerships
- Non-personalised cell therapies: new blood bank model
Public Collection Programmes

Cord Blood Factory

Private Services

Allogeneic Bone Marrow Register

BMT units:
  - CBT
  - DLI

CordPharm

Academia and Industry:
  - Research Bio-resource
  - Non-personalised Cell Therapies
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The role of perinatal care providers


Fox NS, Stevens C, Ciubotariu R, Rubinstein P, McCullough LB, Chervenak FA.
Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, USA.

Women are poorly informed about cord blood banking. The decision making process should be conducted with the goal of ensuring every pregnant woman the opportunity to make a well informed decision about cord blood banking.

The women’s dilemma

To transfer or to maintain ownership?

But...

Universal access to private donation but 2000€
Reduced access to public donation (<1% population)
New requirements for Women Services

• Integration of cord blood donation as a service in a Women’s Service: new role for Obstetricians and Midwives.

• Model:
  - Public vs private
  - Workload
  - Safety: In utero vs Ex utero
  - Requirements in terms of Equipment, HRs and Training

• License for procuring:
  - Private banking: service fee
  - Public banking: Institutional funding/third party agreement
Cord blood donation: activities

Education
Training
Informed Consent
Collection
Non cryopreserved Storage
Non cryopreserved Transport
Follow up
Close Single

Capital, €0.15M:
- HRs: 2
- IT
- Collection trolley
- Blood bank
- Transport

High collection profile
Low repertoire, low thresholds
Low growth
Close Multiple: CONCORDIA model

Intermediate collection profile
Good repertoire, high thresholds
Intermediate growth

Funding?
Open: new public/private partnerships

- Similar to adult bone marrow registers
- General campaign
- Access to any mother interested in public donation
- Require explicit collaboration Perinatal Care Provider
- Potentially funded by donor (Virgin Health Bank model or Stemcyte model) as a service or as a gift

Low collection profile
Good repertoire, low thresholds
High growth

Funding: private by fees (~€2,000)
public maybe ~€200 (coll+transp)
Public Collection Programmes

Private Services

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BMT units:
  CBT
  DLI

CordPharm

Academia and Industry:
  Research Bio-resource
  Non-personalised Cell Therapies
The Anthony Nolan Cell Therapy Centre

Factory (reception, processing, storing, dispatch)

Lab (safety, identity, potency)

Quality (Quality Control and Quality Assurance)
Towards a Cell Factory Concept:
Critical issues

1. Fresh storage and transport
2. Automation
3. Testing: Safety/Identity/Potency
FACILITY

- 200 m² plant-building, three floors: factory, laboratory, technical plant
- 106 m² for Production, 73 m² for quality control and 83 m² for administration

Capacity: 24,000 units
• 1 research tank
• 1 permanent quarantine
• 3 clinical tanks

Productivity:
2000/ per shift
1 Manager
3 Supervisors
4 Technicians

Fix costs: €0.5M/year
Reception
Distribution
Manufacturing
Cryopreservation
Long-term storing
PROCESS

Closed system
- Volume reduction: SEPAX (Biosafe)
- DMSO addition: CoolMix (Biosafe)
- Freezing: CRF (controlled) and LN$_2$ tanks.

Traceability
- bar codes & readers
- Cryobite software: LN$_2$ tanks

Anthony Nolan Cord Blood Database
<table>
<thead>
<tr>
<th><strong>Product Characteristics</strong></th>
<th><strong>Testing</strong></th>
<th><strong>Sample (Type and Timing)</strong></th>
<th><strong>Results of Product Testing</strong></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. (Cytomegalovirus (CMV) results are recorded).</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>Cord blood * and HPC-C (precryopreservation) **</td>
<td>CMV - Report</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin</td>
<td>Cord blood *</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No homozygous hemoglobinopathy</td>
</tr>
<tr>
<td>Purity and Potency</td>
<td>Total nucleated cells (TNC)</td>
<td>HPC-C (precryopreservation)</td>
<td>$\geq 5.0 \times 10^6$ TNC ***/unit HPC-C</td>
</tr>
<tr>
<td></td>
<td>Viable nucleated cells</td>
<td>HPC-C (precryopreservation)</td>
<td>$\geq 85%$ viable nucleated cells</td>
</tr>
<tr>
<td></td>
<td>Viable CD34+ cells (flow cytometry)</td>
<td>HPC-C (precryopreservation)</td>
<td>$\geq 1.25 \times 10^6$ viable CD34+ cells ****/unit HPC-C</td>
</tr>
<tr>
<td>Identity</td>
<td>Human leukocyte antigen (HLA) Typing</td>
<td>Cord blood</td>
<td>Report</td>
</tr>
<tr>
<td></td>
<td>Confirmatory HLA typing</td>
<td>Attached segment of HPC-C</td>
<td>Confirms initial typing</td>
</tr>
<tr>
<td></td>
<td>Blood Group and Rh Type</td>
<td>Cord blood</td>
<td>Report</td>
</tr>
</tbody>
</table>
Releasing tests

Potential risks:

• Safety
• Identity
• Potency

What to do:

• Built quality in front
• Verify: releasing tests

Safety:
- Serology on cord blood

Identity:
- HLA
- Blood group and gender
- Maternal haplotype

Potency:
- CFU/CD34/CLONE
- Viability
- NC recovery
- Volume
PREDICTIVE UTILITY OF THE ATTACHED SEGMENT IN THE QUALITY CONTROL OF A CORD BLOOD GRAFT.

CD34

\[ Y = 0.930 \times + 0.008, \quad R^2 = 0.85 \]

CFU

\[ Y = 0.540 \times + 29.130, \quad R^2 = 0.78 \]

CLONE

\[ Y = 0.711 \times + 10.31, \quad R^2 = 0.83 \]

Rodriguez L, Garcia J and Querol S. Biol Biol Blood Marrow Transpl 2005
Post-thaw CD34+ and CD3+ Cell Viability of Winning-Losing Unit Pairs (N=34 patients, 68 units)

<table>
<thead>
<tr>
<th>Patients with Engraftment (N = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winning units (N = 33)</td>
</tr>
<tr>
<td>Losing units (N = 33)</td>
</tr>
<tr>
<td>Non-engrafting units (N = 2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Winning Unit</th>
<th>Losing Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 75%</td>
<td>&lt; 75%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Winning Unit</th>
<th>Losing Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 75%</td>
<td>≥ 75%</td>
<td></td>
</tr>
</tbody>
</table>
CD34+ CELLS FROM CORD BLOOD UNDERGO APOPTOSIS IN CERTAIN CONDITIONS
Public Collection Programmes

Private Services

Cord Blood Factory

Allogeneic Bone Marrow Register

BMT units:
- CBT
- DLI

CordPharm

Academia and Industry:
- Research Bio-resource
- Non-personalised Cell Therapies
GERMAN ADULT INVENTORY: ~3,000,000

Searches duration for German patients

- % 2 months (urgent): 50%
- % 4 months (non-urgent): 75%

Cord blood programmes are no critical

1 unrelated TX /100000 inhabitants

~5% from cord blood

Source: Annual report 2004-2006, ZKRD
Spain (~60,000 adult donors): probability to find a donor 7 or 8 out of 8

- % 2 months (urgent): 25%
- % 4 months (non-urgent): 45%

Cord blood programmes are critical

0.5 unrelated /100000
40% from cord blood
TOWARDS AN EQUITABLE ACCESS TO A TIMELY THERAPY

The complementary approach

- Sibling: 1/100000
- Unrelated: 1/100000
- Target: 3/100000

HLA restriction

- 4/6: Acceptable
- 5/6: Optimal
- 6/6: Inclusive

- Placental Type: thousands
- Adult Type: millions

National sufficiency
International collaboration
Graft Identification Advisory Service (GIAS)

Objective: 100% transplants on time
Impact of cell dose and HLA Match on survival. Data presented by Dr Pablo Rubinstein at the 5th International Umbilical Cord Blood Symposium held in Los Angeles in May 2007.

Survival

Cell Dose (× 10^7/kg)

5/6 match

4/6 match

How big should the cord blood bank be?

Probability to find at least 1 HLA-A,B low and DRB1 high match

One road, three stations:
- Institutional bank: 4/6; 10,000; 90%
- National programme: 5/6, 50,000; 80%
- International network: 6/6; 1,000,000; 70%

Querol et al, Haematologica in press
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Cord blood bank model: the classical blood bank

- Development of minimally manipulated cord blood components
- Liaison with Biotech companies for advanced cell therapies

CordPharm by-products

**CELLS:**
1. CBMC
2. PROGENITOR CELL POOLS
3. T REG POOLS
4. T EFFECTOR ALLODEPLETION
5. NK ALLOREACTIVE CELLS

- CBMC eMNC (Biosafe) vs Manual Ficoll (Laura)
- CD133 selection (Roger, Richard)
- CD25 selection (Daniel, Richard)
- CD137 vs CD25 depletion (Jim)
- CD3 depletion and NK expansion (Mehri)

**HAEMODERIVATIVES:**
1. PPP
2. PRP
3. RBC (Foetal Hgb)

- MALDI (Sergio)
- MSC isolation and expansion (pendent)
- Marta
RESEARCH IN A CORD BLOOD BANK

• The basis of our research is finding what is unique in cord blood and then test their abilities for therapy

• Therefore, our research model is to assess the differences between the blood components of newborns vs adults by:
  - Pheno(geno)typyc characterisation
  - Functional assays

• Then, the bank acts as catalyst for translational research linking with scientists working in cell therapy and providing clinical grade products for their pre-clinical work
The Anthony Nolan Research Institute

Cellular Immunotherapy Group

Prof Alejandro Madrigal, Dr Sergio Querol
Predicting function using a flow cytometry modified ISHAGE strategy to enumerate CD34: annexin V-7AAD co-staining

Richard Duggleby PhD
ISHAGE gating of stem cells favors the enumeration of live cells

Thawing the same sample shows that the ISHAGE gate favors live cells. But whilst dead cells might be removed there still maybe apoptic cells
Use of Annexin V to further characterize the ISHAGE gated population

T=2 days

Following Fresh cord blood samples with time shows how the stem cell population can become apoptic whilst still having a low 7AAD count.

T=7 days
Frozen cords can have a high degree of variability.

Assessment with Annexin V and 7AAD of the ISHAGE gated stem cells reveals that there can be a significant number of apoptic cells present post thaw of cord units.

Transplanted cord

Successful engraftment

An 100012

Mid viability frozen cord

An 100147

Low viability frozen cord
Viable stem cell assessment predicts the CFU of a sample

Assessment of stem cell numbers in fresh cord blood, frozen CBMC and and frozen cord blood samples; Stem cell numbers determined using ISHAGE gating (with 7AAD) and adjusted using assessment utilizing Annexin V staining as well.

Recent evidence correlates CFU of cord samples with engraftment.

Here, by adjusting the ISHAGE gated stem cell count using the viable stem cell count assessed we can better predict the CFU.
High purity and yield of natural Tregs from cord blood using a single step selection method

Dr Daniel Figueroa-Tentori
New method

P=0.03

P=0.01

P=0.007

% from the starting cell count

Std Method

New Method
Gated on CD4+ cells

MidiMacs isolation

CD4

CD25

CD127

[Graphs and data points indicating CD4, CD25, CD127 distributions and gating areas]
Phenotype post-isolation

90% correlation with FOXP3+ gating on CD25^{high} CD127^{low}
Seddiki, N. J. Exp. Med. 2006; 203:1701
Tregs repertoire

Pool

Ag specific

Polyclonal expansion

Less cells needed
Auto or donor specific
Known Ag
Skewed repertoire

FOXP3 demethylation

Practical
↑ starting cell count
Third party
Unknown Ag
Expand if needed
No need of Rapamycin

Auto or donor specific
Time consuming
Unknown Ag
Maintain starting repertoire
Adult = Rapamycin
CliniMACs isolation of CD25+ CB pools: product characteristics and functional assessment

Richard Duggleby PhD
Purity of CliniMACS isolations

- Initial CD25+ isolations (COM5) were heavily contaminated with CD127hiCD25+ effector cells; giving a low Treg to effector T cell ration in the final product.

- By using lessons learnt using the MidiMACS columns we were able to translate method changes (in press) to the CliniMACS.
  - Despite the increased purity still 6-11x10⁶ cells
Cord CD25+ cells isolated using CliniMACS Ab can suppress a polyclonal stimulus when in combination

Cord CD25+ cells isolated on the MidiMACS have comparable purity.

Initial experiments (needs repeating).

One of the cords responded poorly to inspector beads but in combination both responded and were suppressed by individual or combined CD25+ cells.

Need to repeat using the CliniMACS
Treg function +1 day post isolation

After O/N transportation cord CBMC-25 responded poorly to inspector beads and thus only a trend of suppression is seen by cord CD25+ cells.

Also poor suppression of adult PBMCs responses to beads was also observed; cord CD25+ cells have in the past suppressed bead adult PBMC responses.
Allodepletion of Cord T cells with CD25 / CD137
CD25- CBMCs as a source for DLI

The Anthony Nolan Cord Blood bank is working on providing CD25 + Tregs as a therapeutic resource for the suppression of GvHD.

The CD25- CMBCs could be used as a source for DLI with the prior selected CD25 T regs added back to the DLI after allo depletion.

Working with CD25- CBMCs as a starting resource could enable the use of CD25 as a target for allo depletion.
CD25 / CD137 of CD25 depleted CBMCs in MLR with PBMCs.
CD25/CD137 expression in proliferating cord T cells

CD3/CD28 Dynal bead stimulation of CFSE labeled CD25 depleted CBMCs.
Cord blood NK cells

Mehri Daryouzeh
CD56 CD16 expression in isolated CBMCs
NK cells
Cord Blood CD16+56− cells are possible precursors of mature natural killer cells or a new lineage of NK cells?

- CD16−CD56+ CD117 bright CD94 + NKp30 + 2B44 +
- CD16−CD56+ CD117 bright CD94 + NKp30 + 2B44 +
- CD16+CD56+ CD117 dim CD94 + NKp30 + 2B44 +
- CD16+CD56− CD117− CD94 dim NKp30 dim 2B44 +
1 billion cells?

Clinical

Accredited process

~€400

~€150

~€400

40%

€380,000

600 million cells?

Research

Basic clinical grade

~€150

~€400

40%

€60,000

1000 units collected, €530,000 ~10%

Reagent

Increasing efficiency:

- all units collected, used
- research supply
- new applications

-- low extra investment

2nd generation of CBB

20%
Susana Garcia Gomez
Robert Davy
Roger Horton
Laura Fry
Daniel Gibson
Salmah Mahmood
Hazel Forde
Richard Duggleby
James Devitt
Daniel Figueroa-Tentori
Lia Zambetti
Mehri Daryouzeh
Prof Tony Dodi†
Dr Sergio Querol
Prof John Goldman
Prof Alejandro Madrigal
Dr Nichola Cooper (ICH)
Prof John Martin (UCL)
Prof Manuel N Fernandez (CPH, Madrid)
Prof Robert Rees
Prof Ghulam Mufti
Dr Joan Garcia
Dra Marta Torrabadella
ONE BIRTH, MULTIPLE LIFES

Thank you for your attention