CORD BLOOD BANKING AND
TRANSPLANTATION FOR PATIENTS WITH
INBORN ERRORS OF METABOLISM

Joanne Kurtzberg, MD
Duke University Medical Center
CBU PRODUCT CRYOBAG
How does SCT correct inborn errors of metabolism?

- Marrow and immunoablation
- Replacement with donor cells
- Donor leukocytes produce enzyme
- Enzyme distributed through blood circulation
- Cells migrate to brain, cross blood brain barrier, replace enzyme in brain “Cellular Enzyme Replacement Therapy”
- Non-hematopoietic cell engraftment
Diseases Treated

Krabbe Disease
Metachromatic Leukodystrophy
Adrenoleukodystrophy
Mucopolysaccharidoses
  Hurler, Hunter, Sanfillipo
Neimann Pick Disease
Maroteau Lamy
PMD
Batten Disease
Others
GvHD Prophylaxis and Supportive Care

Preparative Regimen: Bu/Cy/ATG
GvHD Prophylaxis: CYA/Steroids
Supportive Care:
  – Empiric antibiotics with fever
  – Antifungals, Antivirals, PCP prophylaxis
  – IVIG
  – G-CSF
  – TPN
  – Transfusions (14 PRBC, 50 Platelets)
  – LD heparin for VOD prophylaxis
  – PCA
MA chemotherapy is required for engraftment
Two Brothers Transplanted for Late Infantile Krabbe Disease

UCBT age 11 months

UCBT age 2.5 months
Impact of Performance Status on Survival
Coarse Facial Features
Courtesy of E. Krakis, M.D.
SCT for Krabbe Disease: Early transplantation is critical!

Overall Survival by Krabbe Status

Newborn Screening:
New York State 2008
Now 7 other states

Escolar et al, NEJM, 2005
Some newborns with Krabbe Disease have sustained prenatal damage to their cortical spinal tracks.
Donor Cells engraft in the brain after IV UCBT

DUOC-01
Robertson CT²

Stem Cell Lab

CCBB

GMP

Animal models

Clinical Trials

RP2

CT²

Regulatory / QSU

>100 employees
Pathway for the development of DUOC-01

- Hypothesis based on observations in the clinic
- GMP Manufacturing – optimization and validation
- IND - enabling, Preclinical studies
  - Safety
    - Tumorigenicity
  - Mechanisms of action
- Biodistribution
- Release criteria
- Stability
- Clinical protocol development
- IND submission and responses to clinical holds
- IRB submission including review by the SCRO
- Study monitoring and data analysis
DUOC-01 (O-cells)

• R & D:
• Manufacturing Validated
• Characterization: CD45++, 11b++, 01/04+
• IND submitted and approved
  – Additional preclinical studies performed
  – Biodistribution
  – Efficacy
• IRB approved for IT administration in LSD
• 1st patient treated 2/20/2015
• Planned 6-18 patients
**Figure 1. CFSE staining.**

Two different DUOC-01 cell preparations were modified with 2 μM CFSE prior to their transplantation into neonatal NSg mice. As a control, some of these cells were placed in culture for 7 days to monitor the level of fluorescence that is maintained by the cells during that period of time.

*Upper panel:* The level of background fluorescence in unlabeled cells.

*Middle panel:* The level of fluorescence of DUOC-01 immediately following the CFSE labeling. This panel demonstrates fluorescence of the cells at the time when the mice were transplanted.

*Lower panel:* The level of fluorescence maintained by the cells following 1 week in culture. This panel demonstrates the potential fluorescence of the cells at the time when the mice were sacrificed.
Figure 3. Detection of human cells within murine brain.
For this study, tiled files of contiguous fields were acquired and merged as a composite image. Each composite typically represented between 16 and 25 individual images. Each image was acquired at 40x magnification.

*Left panel:* A single representative individual image is shown. In this field, human cells demonstrated green fluorescence as well as staining for the human nuclear antigen (shown in red). The nuclear antigen was coincident with staining for nuclear DNA (shown in blue).

*Right panel:* One composite image taken from the cerebellum of a transplanted mouse is shown. A yellow box indicates the origin of the field represented in the Left panel.
DUOC-01 First in Humans Trial Design

80%

CBT

20%

IT 1-5 X10^6 cells

DUOC-01

21 days

Cells from CBT in CNS

Period DUOC-01 will provide benefit

Assess: Safety, Motor function, Cognitive function, long term

IND 9/2014
Our Roadmap

1. Allo UCBT in IMD
2. Donor cells engraft in brain
3. Further injury prevented, some repair
4. What about auto cells for brain injury?
5. What about an allo cord-derived product for Brain Injury?

Questions:
- What about auto cells for brain injury?
- What about an allo cord-derived product for Brain Injury?
Autologous UCB Trials at Duke

- Safety
  - Cryopreserved UCB
- HIE Study “Babybac”
  - Fresh, VR, RR, CUB
- Congenital Hydrocephalus
  - Multiple doses of UCB
- HLHS/ECMO
  - Fresh and cryopreserved
- CP
  - Cryopreserved
- Autism
  - Cryopreserved
Differences in quality between privately and publicly banked umbilical cord blood units: a pilot study of autologous cord blood infusion in children with acquired neurologic disorders

Jessica Sun, June Allison, Colleen McLaughlin, Linda Sledge, Barbara Waters-Pick, Stephen Wease, and Joanne Kurtzberg
Autologous UCB in Children with Acquired Neurologic Disorders – Phase I

- 184 patients; 198 infusions
  - Median 2.3 years
- CP 76%, congenital hydrocephalus, other injuries
- Minimum requirements for CBUs
- Outpatient infusions, PIV
- 3 safety events, all resolved + 1 in Mom
- Efficacy difficult to evaluate
- Quality parameters assessed
Multiple dosed auto UCB infusions in babies with congenital hydrocephalus

• Study period: 2006-2014
• Patient Characteristics
  – 72 patients, 132 % infusions
  – Gender: 37 male, 35 female
  – Age: median 2 months at time of first infusion, range 6 days to 4.5 years
  – Etiology:
    – 49% Aqueductal stenosis
    – 12% IVH/stroke
    – 39% Other
    – One patient subsequently found to have a genetic syndrome
Time Course

<table>
<thead>
<tr>
<th>Event</th>
<th>N</th>
<th>Median Age</th>
<th>Minimum Age</th>
<th>Maximum Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>72</td>
<td>3 days</td>
<td>0 days</td>
<td>10 mos</td>
</tr>
<tr>
<td>Shunt Placed</td>
<td>64</td>
<td>2 mos</td>
<td>6 days</td>
<td>4.5 years</td>
</tr>
<tr>
<td>1st Infusion</td>
<td>72</td>
<td>9 mos</td>
<td>2 mos</td>
<td>3.6 years</td>
</tr>
<tr>
<td>2nd Infusion</td>
<td>40</td>
<td>15 mos</td>
<td>7 mos</td>
<td>3.2 years</td>
</tr>
<tr>
<td>3rd Infusion</td>
<td>16</td>
<td>16 mos</td>
<td>10 mos</td>
<td>1.9 years</td>
</tr>
<tr>
<td>4th Infusion</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Is there a role for UCB infusions in children with Autism?

- **Phase I Endpoint Finding Trial: Patient population**
  - ASD
    - ages 2-6 years
    - IQ >35

- **Timing**
  - 1 infusion with f/u 3/6/9/12 months

- **Endpoints**
  - TBD in first 25 patient open-label study

- **Mechanisms of action**
  - Paracrine signaling which signals endogenous cells to increase neural connectivity (EEG/MRI)
  - Exploring in the Shank-3 murine model
Autism and CP rPh II: Trial Design

Randomize

Evaluation

Infusion 1
- Placebo
- Best Donor Source
  - Auto
  - Allo

Infusion 2
- Best Donor Source
  - Placebo
  - Auto
  - Allo

Year 1
Year 2
Human Umbilical Cord Blood Cells Ameliorate Motor Deficits in Rabbits in a Cerebral Palsy Model

Alexander Drobyshhevsky\textsuperscript{a}  C. Michael Cotten\textsuperscript{b}  Zhongjie Shi\textsuperscript{a}  Kehuan Luo\textsuperscript{a}  
Rugang Jiang\textsuperscript{a}  Matthew Derrick\textsuperscript{a}  Elizabeth T. Tracy\textsuperscript{b}  Tracy Gentry\textsuperscript{b}  
Ronald N. Goldberg\textsuperscript{b}  Joanne Kurtzberg\textsuperscript{c}  Sidhartha Tan\textsuperscript{a}

\textsuperscript{a}Department of Pediatrics, NorthShore University HealthSystem, Evanston, Ill., and \textsuperscript{b}Department of Pediatrics and \textsuperscript{c}Robertson Cell and Translational Therapy Program, Duke University, Durham, N.C., USA
Peripheral infusion of HUCB cells or media or saline after birth at E31.

- 1.0 ml HUCB cells obtained from JK's lab, $5.0 \times 10^6$/ml, or 1 ml media or saline
- Injection via external jugular vein or abdominal wall vein; 4 hrs after C-section
- Determination of Mild and Severe Groups made at E31.
MOTOR FUNCTION AFTER CORD BLOOD

Intrauterine hypoxia e22
C/S e31
Severe phenotype
4 hours postnatal
2.5.0x10^6/ml saline IV

HUCB Cells improve Motor Function in rabbit CP model.

Replication
• same volume (1 ml)
• lower dose (2.5 x 10^6)
• Compared cells to media+saline
• Improved outcome
• No increase in mortality

In Vitro: Brain slice model: Representative OGD data

1h Control- No OGD

1hr post OGD

72-hr post OGD

72h Control- No OGD

72h post OGD CB CD14 (+)

72h post OGD CB CD14-depleted

GFAP, NeuN, Iba1
Feasibility of Autologous Cord Blood Cells for Infants with Hypoxic-Ischemic Encephalopathy

C. Michael Cotten, MD¹, Amy P. Murtha, MD², Ronald N. Goldberg, MD¹, Chad A. Grotegut, MD², P. Brian Smith, MD¹, Ricki F. Goldstein, MD¹, Kimberley A. Fisher, PhD¹, Kathryn E. Gustafson, PhD³, Barbara Waters-Pick, BS, MT(ASCP)⁴, Geeta K. Swamy, MD², Benjamin Rattray, MD¹, Siddhartha Tan, MD⁵, and Joanne Kurtzberg, MD⁶

HIE (babybac) Pilot Study
Mike Cotten, Ron Goldberg, Amy Murtha, STCL, CCBB

• Term Newborns with HIE meeting diagnostic criteria for moderate to severe encephalopathy
• Eligible for cooling
• Collected autologous cord blood
• Cooled per SOC
• Informed consent
• Given autologous CB infusions at <24 and <48 hours of age
• Followed for infusional toxicity, survival and functional outcomes at 1 and 2 years of age

Cotton et al, J Peds, 2014
Inclusion Criteria

- **Is cord blood available?**
- HIE evaluation: Two step process:
  - Step A: clinical/biochemical criteria
  - Step B: neurological examination
- Evaluate infants for:
  1. Acute perinatal event (abruption, cord prolapse, severe FHR abnormality)
  2. Apgar ≤ 5 at 10 min
  3. Ventilation at birth and continued for a minimum of 10 min
  4. Cord pH or any postnatal pH at ≤ 1hr ≤ 7.0
  5. Cord base deficit or any postnatal BE at ≤ 1hr ≥ 16mEq/L

Collection
Transport to prep site
Transport back to ICN
Infusion
- Baby born, in distress;
- OB obtains verbal assent to collect CB;
- CB collected, mom consented for study;
- CB processed into 4, 5ml aliquots;
- Baby dosed daily x 4 (<6h, 24h, 48h, 72h).
## Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cell recipients Mean (min/max) or N (%) N = 45</th>
<th>Concurrent cooled Mean (min/max) or N (%) N = 154</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age, wks</td>
<td>39 (34/41)</td>
<td>39 (33/41)</td>
<td>0.88</td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>3330 (2120/4660)</td>
<td>3300 (1770/4872)</td>
<td>0.78</td>
</tr>
<tr>
<td>SGA</td>
<td>2 (4)</td>
<td>13 (8)</td>
<td>0.53</td>
</tr>
<tr>
<td>LGA</td>
<td>4 (9)</td>
<td>15 (10)</td>
<td>0.99</td>
</tr>
<tr>
<td>C-section delivery</td>
<td>35 (78)</td>
<td>92 (60)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Outborn</strong></td>
<td><strong>15 (33)</strong></td>
<td><strong>113 (73)</strong></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Males</td>
<td>21 (47)</td>
<td>98 (63)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
## Subject Characteristics

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<tr>
<td></td>
<td>N = 45</td>
<td>N = 154</td>
<td></td>
</tr>
<tr>
<td>5 minute Apgar ≤ 5</td>
<td>37 (82)</td>
<td>110 (72)</td>
<td>0.18</td>
</tr>
<tr>
<td>10 minute Apgar ≤ 5</td>
<td>22 (50)</td>
<td>71 (53)</td>
<td>0.73</td>
</tr>
<tr>
<td>Cord pH</td>
<td>6.98 (6.50/7.27)</td>
<td>6.99 (6.48/7.44)</td>
<td>0.46</td>
</tr>
<tr>
<td>Cord pH &lt; 7</td>
<td>26 (59)</td>
<td>79 (54)</td>
<td>0.60</td>
</tr>
<tr>
<td>Base deficit ≥ 16</td>
<td>27 (60)</td>
<td>60 (39)</td>
<td>0.16</td>
</tr>
<tr>
<td>NICHD score*</td>
<td>21 (12/49)</td>
<td>20 (5/59)</td>
<td>0.65</td>
</tr>
<tr>
<td>NICHD score* ≥ 30</td>
<td>8 (18)</td>
<td>34 (22)</td>
<td>0.68</td>
</tr>
<tr>
<td>Seizures</td>
<td>15 (33)</td>
<td>71 (47)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

### Feasibility Measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean or N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal consent for cord blood collection</td>
<td>Approx 2/3rds</td>
</tr>
<tr>
<td>Volume Collected, ml</td>
<td>39 (3, 178)</td>
</tr>
<tr>
<td># Cells post processing (x 10^8)</td>
<td>8.6 (0.77, 35)</td>
</tr>
<tr>
<td>Time to first infusion</td>
<td></td>
</tr>
<tr>
<td>≤ 6 hr</td>
<td>25 hr (3.9, 220)</td>
</tr>
<tr>
<td>&gt; 6 hr</td>
<td>5 (11)</td>
</tr>
<tr>
<td></td>
<td>40 (89)</td>
</tr>
<tr>
<td>Number of infusions</td>
<td></td>
</tr>
<tr>
<td>4*</td>
<td>11 (25)</td>
</tr>
<tr>
<td>3*</td>
<td>3 (7)</td>
</tr>
<tr>
<td>2</td>
<td>20 (45)</td>
</tr>
<tr>
<td>1</td>
<td>8 (18)</td>
</tr>
<tr>
<td>0</td>
<td>2 (1 severe chorio, 1 ECMO)</td>
</tr>
<tr>
<td>Infusion volume ml</td>
<td>5 (1, 10)</td>
</tr>
</tbody>
</table>

## Hospital Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Cell recipients</th>
<th>Concurrent cooled</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 45</td>
<td>N = 154</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECMO</td>
<td>5 (11)</td>
<td>9 (6)</td>
<td>0.32</td>
</tr>
<tr>
<td>Deaths</td>
<td>1 (2)</td>
<td>18 (12)</td>
<td>0.08</td>
</tr>
<tr>
<td>Seizure meds at discharge</td>
<td>10 (23)</td>
<td>32 (24)</td>
<td>0.99</td>
</tr>
<tr>
<td>100% Oral feeds at discharge</td>
<td>34 (77)</td>
<td>110 (80)</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Survival with 1 yr Bayley III scores \( \geq 85 \) in 3 domains

<table>
<thead>
<tr>
<th></th>
<th>Cells N = 28 N (%)</th>
<th>Cooled only N = 66 N (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival with all 3 Bayley domain scores ( &gt; 85 )</td>
<td>18 (64)</td>
<td>25 (38)</td>
<td>0.04</td>
</tr>
<tr>
<td>Bayley &lt; 85 at one year (among survivors)*</td>
<td>9 (35)</td>
<td>23 (48)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

* 2 cell recipients died after discharge

**Multivariable logistic regression for mortality or Bayley III scores < 85:**
- Cells compared with no cells: 0.34; 95% CI 0.12, 0.94.
- Higher NICHD score OR 1.11; 95% CI 1.04, 1.18
“CP-AC” (IND)

- Randomized, placebo-controlled trial of autologous CB in children with spastic CP
  - Ages 1-6 yrs
  - Eligible cord blood
  - GMFM level (II-IV)
- Blinded/cross-over design
  - Baseline, 1 yr, 2yrs
- Evaluations by exam, neurocog/fxnl testing, MRI (functional in older pts), TMS, CB microarrays, QOL
- Primary Endpoint: >30% increase in predicted GMFM score at 1 year; Secondary Peabody Motor Score
- Activated 7/2010; completed accrual 2/2013
- Last patient reached primary endpoint 2/2014
- Analysis planned by EMMES 4/2015
Enrolled on Study → RANDOMIZED → Arm 1 → UCB → Visit 1 (time 0) → Evaluation

Arm 1 → Placebo → Visit 2 (1st year) → Evaluation

Arm 2 → Placebo → Visit 2 (1st year) → Evaluation

Enrolled on Study → RANDOMIZED → Arm 2 → Placebo → Visit 2 (1st year) → Evaluation

Enrolled on Study → RANDOMIZED → Arm 1 → UCB → Visit 3 (2nd year) → Evaluation

Enrolled on Study → RANDOMIZED → Arm 2 → Placebo → Visit 3 (2nd year) → Evaluation

*Placebo = TC199 + 1% DMSO
GMFM: Assessing Change in Changing Subjects

Assumptions:

- Mean increase of 6 points/year without intervention
- ~30% additional increase (7.8 total points/year) would be clinically significant
GMFM change < 10

- Increased normalized connection volume
- Decreased normalized connection volume

GMFM change >= 10

- cp010 (T(Left UE))
- cp011 (H(Lh))
- cp009 (H(Rh))
- cp025 (Q)
- cp002 (Q)
- cp005 (Q)
Allogeneic cells for Acquired brain injuries and other cellular therapies?

• Most patients do not have their cord blood banked.
• A donor derived, readily available product is needed:
  – Administration without chemotherapy.
  – Will immunosuppression be needed?
  – Should the product be HLA matched?
• Therapeutic effects through paracrine signaling
• Durable engraftment not necessary
Approach: Clinical Development

- Autologous Children
- Allogeneic Safety Adults
- Allogeneic Efficacy Adults
- Expectation of benefit in Children
- Allogeneic Children
Phase I Allo UCB Infusion in adults with sub-acute stroke

- Ischemic Stroke
- Days 3-10
- Off the shelf UCB Unit infused IV
- No HLA matching
- ABO/Rh and Race matching
- Primary endpoint – safety
  - PRA
- Secondary endpoints – clinical efficacy at 3 months, 6 months
- IND approved 12/2014
- Plan for follow-on phase II randomized study
25 Minute MCAO Infarct Volume

Cord Blood versus Vehicle

Infarct Volume on Day 10

Cord Blood

Vehicle

Infarct Volume (mm³)

0
10
20
30
40
Effect of Cord Blood Infusion on survival after MCAO

Young adult male C57-BL6 mice received stroke (25 minutes of middle cerebral artery ischemia/reperfusion) on day 0.

Cord blood or vehicle infused intravenously on day 5 post-stroke.

Significant mortality difference on day 10.

Days after stroke:
- Treatment on Day 5
- 83% survival for Cord blood
- 50% survival for Vehicle
Post-Stroke function is improved after cord blood treatment

Neuroseverity Score, Day 10 after Stroke

<table>
<thead>
<tr>
<th></th>
<th>Cord Blood</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSS</td>
<td>10±2</td>
<td>15±3</td>
</tr>
</tbody>
</table>
UCB Allo Stroke: Updated Trial Design

Phase I
N=10
- No match
- Day 3-10
- IV

Phase II Randomized
N=80
- Placebo
- N = 40

Phase I
N=10
- No match
- Day 3-10
- IC

IV
N = 40
Where are we now?

Safety of autologous UCB infusion

- 184 children, 198 infusions
- 72 children with CH treated with 2-4 sequential infusions
- HIE (45 patients)
- Autism (25 patients)

Efficacy of auto UCB infusion (n=160)

- HIE
- CP
- Autism

Safety of allo UCB infusion

- Adult stroke
Where are we going?

Randomized Phase II placebo controlled efficacy trials

**Specialized cells**
- DUOC-01
- CD14 UCB
- UC (WJ) MSCs

**Children**
- HIE (160)
- CP (160)
- Autism (160)

**Adults**
- Adult stroke
  - (10+80)
Research Laboratory goals for all cell products

- Proof of concept in animal and culture models: Efficacy & delivery
- Mechanism of action studies
- Proof of manufacturing feasibility
- Transfer manufacturing & bioassay technology to cGMP
FDA LICENSURE
‘hematopoietic reconstitution after myeloablative chemotherapy’
“It takes a village......”

Pediatric Blood and Marrow Transplant Team
- MDs, APNs, NCs, SC, SW, FA, FSP

Stem Cell Laboratory

Carolinas Cord Blood Bank

CT2: Andy Balber and team
- EJ Shpall, Mike Frankel
- Allen Song and Jim Provenzale
- Jessica Sun/Mohamad Mikati/Gordon Worley
- Katie Gustafson/Laura Case and ND Team
- Amy Murtha, Haywood Brown
- Sid Tan, Mike Cotten, Ron Goldberg
- Geri Dawson and team
- Danny Laskowitz and team

NHLBI, HRSA, NMDP, The EMMES Corp

The Julian Robertson Foundation

The Legacy of Angels Foundation

The Katz Foundation

The Marcus Foundation

Our Patients and their parents and families

Hillary Clinton