Hypothermia and Beyond:
Fine Tuning Therapeutic Hypothermia and Evidence for Stem Cells as a Safe and Effective Add-on

C. Michael Cotten MD MHS
November 2019
Disclosures

• Grant support from NICHD for Neonatal Research Network, including clinical trials of hypothermia.
• NICHD for SBIRs for lab-on-chip for hyperbilirubinemia, hypoglycemia, hypothyroid, acute kidney injury, (not brain injury)
• Robertson Foundation: Autologous Cord Blood Cells for HIE phase II
• NCATS: Duke CTSA pilot study: allogeneic human cord tissue derived MSC’s for infants with HIE.
• Advisory board, Mallinkrodt pharmaceuticals: clinical trial development for hyperbili intervention.
Neonatal Encephalopathy

• Disturbed neurologic function in earliest postnatal days in infant born > 35 weeks
  – Subnormal level of consciousness or seizures
  – Frequent difficulty maintaining respiration
  – Depressed tone and reflexes….  
  
  – Perinatal/neonatal Hypoxic-ischemic encephalopathy (HIE) suspected w/ abnormal cord/very early postnatal blood gas, perinatal history, exam, early eeg

Hypothermia for Birth Asphyxia: Accumulating Evidence

“Sarah Parks … gave still-birth to a baby boy …

A young doctor assisting the Parks' regular physician begged for an opportunity to experiment with an idea he had to rouse the lifeless infant.

A tub of ice was ordered and the young doctor plunged the baby into it.

Out came the *screaming* little Parks and he was named Gordon after the doctor who prodded him to life.”—Sir John Floyer, 1697

Wyatt JS, Thoresen M. Hypothermia treatment in the newborn. Pediatrics 1997;100:1028-1030
Goals of the talk

• How did we get from the 17th century to current practice?
• What’s next?
Epidemiology and Burden of HIE - non-anomalous term infants -

• **2.5/1000**: Incidence of HIE live births in high income/low infant mortality countries.

• **15%**: Proportion of cerebral palsy associated with intrapartum hypoxia-ischemia

• **1.15 million babies w/ NE, 96% in low and middle income countries**

• **50.2 million DALYs (2.4% of total) and 6.1 million years living with disability (YLDs) attributed to Intrapartum-related conditions.**
  - More DALY’s than diabetes
  - About 75% of the DALY’s attributed to HIV/AIDS


Cooling asphyxiated neonatal guinea pigs

• 1940’s Standard practice = place asphyxiated newborns in incubators

• BUT: JA Miller, Jr. (Emory) raised questions:
  • Higher temperature increased rates of chemical reactions
  • Van Herrenveld and Tyler (1944) demonstrated that a 10° reduction in temperature was sufficient to protect the spinal cord of cats from damage from asphyxia.

• SO: he questioned the rationale for warming asphyxiated babies and performed experiments to test the effects of temperature upon resistance to asphyxia in the neonatal guinea pig (1949 – 1954)....

Cooler Guinea pigs lived longer...

- N = 205 animals
- 25 animals w/ temps > 43° C or < 19° C died before studies could begin
- Remaining animals placed in 95% N₂/5% CO₂
- Measure time to last gasp at different temps
  - Shortest: 81 sec’s @ 44.2°
  - Longest 617 sec’s @ 14.1°
- There were some survivors!!!
  (adding sedation with sodium pentathol improved survival duration)

X axis: time to last gasp (increasing)
Y axis: temperature (descending)

240 sec 360 sec 600 sec

“This short preliminary account of results of this investigation is being presented at this time in the hopes that it may stimulate clinical studies on the treatment of asphyxiated newborn infants”

10 years later...Early clinical experience

Hypothermia recollections by Bjorn Westin

- 1958 – 1959
- 10 severely asphyxiated infants
  - Apgar 1 at 5 min
  - Avg apnea 17.4 min
  - Immersed cold water, core temps 23 - 28°C
- 9 survived (one died of RDS at 30 hours)
- Follow-up 16 - 24 months: intact.

But concerns had risen: Hypothermia: Increased Risk for Prematures: 1958 - 1964

• Higher mortality if hypothermic in the NICU, or not servo-maintained at 36.5°C

Hypothermia: Doubt in 1966, fetal rhesus monkeys

- c/s delivery at term
- Asphyxiated by tying the cord and covering the head
- Cooled animals placed in cold water bath, controls kept at 30°C air, until last gasp, then BMV;
- No difference in outcome, need for resuscitation
- “We conclude that hypothermia of itself is of little value in the resuscitation of the asphyxiated newborn.”

- One flaw was in thinking cooling = resuscitation

Hypothermia: *more doubt in 1976*

- Animal experiments and clinical recommendations led Oates and Harvey to study *cooling in asphyxiated rabbit pups*...
- Like Miller and Westin, if cooling was rapid and begins early in asphyxia, survival was prolonged,...

### Survival of newborn rabbits asphyxiated for 1 1/2 times the mean time to the last gasp (TLG) of litter mate controls, when hypothermia was started after 1/4, 1/2, and 3/4 way through asphyxia

<table>
<thead>
<tr>
<th>Rate of cooling</th>
<th>Duration of asphyxia before cooling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/4 of 1 1/2 TLG of controls</td>
</tr>
<tr>
<td>Slow (20 °C)</td>
<td>Died</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Fast (10 °C)</td>
<td>9</td>
</tr>
</tbody>
</table>

- **BUT they conclude...** _because humans can’t be cooled as fast as rabbit pups_, “if accepted measures [of resusc] are unsuccessful, there seems to be little advantage in cooling...”

The experts....and emergence of technology...

- 1971: Schaffer and Avery: euthermia
- 1973: Klaus and Fanaroff: euthermia
- **BUT.....discoveries/new technology....lead to more discoveries, and hypotheses....MRS and secondary energy failure**

https://www.britannica.com/science/magnetic-resonance-spectroscopy
1980’s–90’s: MRS technology: Inorganic phosphate rises, Pcr, ATP fall

- $^{31}$P normal in first postnatal hours in term infants with asphyxia
- [$\text{PCr}/[\text{Pi}]$ subsequently decreases, particularly ATP, despite normalized systemic pH (secondary energy failure)
- In this piglet study, mimicking perinatal asphyxia,
  - ATP depletion \textit{w/o acidosis}
  - Brain exams indicated necrosis, apoptosis, and secondary damage associated with cerebral edema.

Mechanisms of Brain Injury in the Term Neonate

Release and accumulation of excitotoxic amino acids: Glutamate

Primary energy failure
- Acute cell death

Secondary energy failure
- apoptosis

Injury timing?

Glutamate targeted

- Adult Animals
- Global ischemia x 20 minutes
- Reduced cerebral blood flow
- Cooling to 30° and 33° C reduced Glutamate release

Hypothermia
By late 90’s: Total Body Cooling in small and large Animal Models

DEPTH
Neuroprotection optimal at 32-34° C
- <32 ° C less neuroprotective
- <30 ° C associated with systemic side effects

DURATION
- Prolonged cooling (up to 72 h) greater protection than brief periods
- Could use slightly less hypothermic for longer time: similar effects

TIME OF INITIATION
- Greatest protection when initiated immediately after the insult.
- Benefit up to 6 hours, after, but not so much after seizures.

https://www.google.com/search?q=babe+pig+and+sheep&rlz=1C1GGRV_enUS748US748&source=univ&sa=X&ved=2ahUKEwiIrpLmrdHjAhVid8KHSuDDRAQ7Al6BAgEECO&biw=1280&bih=610
https://www.google.com/search?q=ratatouille+movie&rlz=1C1GGRV_enUS748US748&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiIrpLmrdHjAhVid8KHSuDDRAQ7Al6BAgEECO&biw=1280&bih=610
Clinical Trials

• 6 phase III’s: CoolCap*, NICHID*, TOBY (Total Body Hypothermia for Neonatal Encephalopathy Trial), ICE (infant cooling evaluation), neo.nEURO, Zhou

*Selective Head Cooling; the rest are whole body cooling studies
Cooling Cap
## CoolCap

### Results: Primary Outcome; unadjusted

<table>
<thead>
<tr>
<th>Death or NDI whole cohort</th>
<th>Cooled (n = 108)</th>
<th>Control (n = 110)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or NDI whole cohort</td>
<td>59 (55%)</td>
<td>73 (66%)</td>
<td>0.61 (0.34-1.09)</td>
</tr>
<tr>
<td>Died</td>
<td>36 (33%)</td>
<td>42 (38%)</td>
<td>0.81 (0.47 – 1.41)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Cooled</th>
<th>Control</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moderate aEEG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died or NDI</td>
<td>40 (48%)</td>
<td>58 (66%)</td>
<td>0.47</td>
<td>(0.26 – 0.87)</td>
</tr>
<tr>
<td><strong>Severe aEEG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died or NDI</td>
<td>19 (79%)</td>
<td>15 (68%)</td>
<td>1.8</td>
<td>(0.49 – 6.4)</td>
</tr>
</tbody>
</table>

NO effect among severe, but ? If moderate group is balanced?

Whole Body Hypothermia for Hypoxic-Ischemic Encephalopathy

Seetha Shankaran, MD

for

The NICHD Neonatal Research Network

Shankaran et al. NEJM 2005; 353:1574-1584
C. Subzero Standard Cooling Unit

Adult cooling blanket; acts as cooling reservoir to avoid swings in blanket temp

Baby on pediatric cooling blanket
**Inclusion Criteria**

Blood Gas

- pH $\leq 7.0$ OR $\text{BD} \geq 16$
- If No Blood Gas OR base deficit between 10 and 15 OR pH between 7 and 7.15 need to have:
  - Acute Event **AND**
  - 10 min Apgar $\leq 5$
  - Ventilation from birth $\geq 10$ minutes

SEIZURES OR MODERATE/SEVERE HIE

Shankaran et al. NEJM 2005; 353:1574-1584
**HIE Exam Criteria (certified examiners)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Moderate Encephalopathy</th>
<th>Severe Encephalopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Level of consciousness</td>
<td>Lethargic</td>
<td>Stupor/coma</td>
</tr>
<tr>
<td>2. Spontaneous activity</td>
<td>Decreased</td>
<td>No activity</td>
</tr>
<tr>
<td>3. Posture</td>
<td>Distal flexion</td>
<td>Decerebrate</td>
</tr>
<tr>
<td>4. Tone</td>
<td>Hypotonia (focal, general)</td>
<td>Flaccid</td>
</tr>
<tr>
<td>5. Primitive reflexes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suck</td>
<td>Weak</td>
<td>Absent</td>
</tr>
<tr>
<td>Moro</td>
<td>Incomplete</td>
<td>Absent</td>
</tr>
<tr>
<td>6. Autonomic system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupils, Heart rate, Respirations</td>
<td>Constricted</td>
<td>Skew deviation/dilated/non-reactive to light</td>
</tr>
<tr>
<td></td>
<td>Bradycardia</td>
<td>Variable HR</td>
</tr>
<tr>
<td></td>
<td>Periodic breathing</td>
<td>Apnea</td>
</tr>
</tbody>
</table>

**Shankaran et al. NEJM 2005; 353:1574-1584**
Infant with HIE
<6 hours of age

**HYPOTHERMIA**
- Eso 33.5°C / 72 hr
- Monitor adverse events
- Rewarm 0.5°C / hr
- Remove eso probe

**NORMOTHERMIA**
- Monitor eso temp 72 hr/skin servo on warmer*
- Monitor adverse events
- Remove eso probe

Shankaran et al. NEJM 2005; 353:1574-1584
Primary Outcome:

Death or Moderate to Severe Disability

Survivors were evaluated at 18 mos by certified examiners

Severe disability: ANY of the following
- MDI < 70
- GMF level 3-5
- Hearing impairment requiring aids
- Blindness

Moderate disability: MDI 85-70 and ANY of the following
- GMF Level 2
- Hearing impairment with no amplification
- Seizure disorder

Shankaran et al. NEJM 2005; 353:1574-1584
Esophageal Temperature

Note the overshoot

Shankaran et al. NEJM 2005; 353:1574-1584
### NRN Whole Body Hypothermia

#### Primary Outcome

<table>
<thead>
<tr>
<th></th>
<th>Hypothermia</th>
<th>Normothermia</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n=102</td>
<td>n=106</td>
<td></td>
</tr>
<tr>
<td>Death or Moderate/Severe disability</td>
<td>45(44%)</td>
<td>64(62%)</td>
<td>0.71(0.54-0.93)</td>
</tr>
</tbody>
</table>

- **NNT=6**

CoolCap: Cooled: 55% died or severe impairment; control 66%

Shankaran et al. NEJM 2005; 353:1574-1584
Survival Curve: NRN Hypothermia Trial Thru Follow-Up at 18 – 22 months

Shankaran et al. NEJM 2005; 353:1574-1584
## RESULTS FOR SUBGROUPS

<table>
<thead>
<tr>
<th></th>
<th>Hypothermia N=102</th>
<th>Normothermia N=106</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>24 (24%)</td>
<td>38 (36%)</td>
<td>0.66 (0.43-1.01)</td>
</tr>
<tr>
<td>Death or disability after <strong>Moderate</strong> HIE (n=132)</td>
<td>22 (32%)</td>
<td>30 (48%)</td>
<td>RR 0.67 (0.44-1.03)</td>
</tr>
<tr>
<td>Death or disability after <strong>Severe</strong> HIE (n=72)</td>
<td>23(72%)</td>
<td>34(85%)</td>
<td>RR 0.85 (0.66-1.09)</td>
</tr>
</tbody>
</table>

(74 severe in cool cap)

Shankaran et al. NEJM 2005; 353:1574-1584
Primary Outcomes
4 Pivotal Whole Body Hypothermia Trials

- 44 – 51% of infants died or survived with disabilities
- 24 - 38% of babies with HIE and were cooled died
- 13 – 28% of the survivors were later diagnosed with cerebral palsy.

Shankaran S et al. NEJM 2005; 353:1574-1584
### Table

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Hypothermia</th>
<th>Normothermia</th>
<th>Risk Ratio M-H, Fixed (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants With Moderate Encephalopathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azzopardi et al,13 2009</td>
<td>20</td>
<td>65</td>
<td>17.9</td>
</tr>
<tr>
<td>Gluckman et al,16 2005</td>
<td>26</td>
<td>62</td>
<td>39</td>
</tr>
<tr>
<td>Gann et al,7 1998</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Jacobs et al,20 2011</td>
<td>26</td>
<td>61</td>
<td>34</td>
</tr>
<tr>
<td>Shankaran et al,12 2005</td>
<td>22</td>
<td>69</td>
<td>30</td>
</tr>
<tr>
<td>Simbruner et al,21 2010</td>
<td>6</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Zhou et al,22 2010</td>
<td>9</td>
<td>41</td>
<td>19</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>327</td>
<td>311</td>
<td>100.0</td>
</tr>
<tr>
<td>Total events</td>
<td>115</td>
<td>162</td>
<td></td>
</tr>
</tbody>
</table>

#### Subtotal (95% CI) 327 311 100.0 0.67 (0.56-0.81)

#### Heterogeneity: $X^2/6=3.75; P=.71; I^2=0%$

**Test for overall effect: $z=4.27; P<.001$**

<table>
<thead>
<tr>
<th>Infants With Severe Encephalopathy</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Azzopardi et al,13 2009</td>
<td>54</td>
<td>98</td>
<td>56</td>
</tr>
<tr>
<td>Gluckman et al,16 2005</td>
<td>28</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>Gann et al,7 1998</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Jacobs et al,20 2011</td>
<td>25</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Shankaran et al,12 2005</td>
<td>23</td>
<td>32</td>
<td>34</td>
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<td>21</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td>Zhou et al,22 2010</td>
<td>22</td>
<td>38</td>
<td>27</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>275</td>
<td>276</td>
<td>100.0</td>
</tr>
<tr>
<td>Total events</td>
<td>175</td>
<td>215</td>
<td></td>
</tr>
</tbody>
</table>

#### Subtotal (95% CI) 275 276 100.0 0.83 (0.74-0.92)

#### Heterogeneity: $X^2/6=5.12; P=.53; I^2=0%$

**Test for overall effect: $z=3.46; P<.001$**

### Figure Legend

Figure 5. Forest plot of the **primary outcome of death or major disability in survivors** in newborns with moderate to severe hypoxic ischemic encephalopathy. Diamond indicates overall summary estimate for the analysis (width of the diamond represents the 95% CI). M-H indicates Mantel-Haenzel test.
Hypoxia-ischemia

Primary injury
- Primary cell death
- Cell swelling

Reperfusion
- Connexin hemichannel opening
- NMDA receptor hyperexcitability
- Mitochondrial collapse

Latent phase
- ~ 6 hours
  - Excitotoxicity
  - Oxidative stress
  - Pro-apoptotic signals
  - Epileptiform transients
  - Inflammation
  - Seizures
  - Cell swelling

Secondary phase
- 6 to 72 hours
  - Cell death
  - Impaired connectivity
  - Impaired maturation

Tertiary phase
- Days to months
  - Loss of trophic support
  - Chronic inflammation

Brain injury

What’s Next for HIE?

• Using Hypothermia most effectively:
  – Who’s being cooled?
  – What’s prognostic
  – What about later childhood outcomes?
  – What about deeper/longer cooling?
  – What if you have to start later?

• Cooling Plus?
  – What’s being tested
  – Cord blood cells: phase I and II (single site); MSC’s phase I
Predicting Poor Outcome?

• Low 10-minute Apgar score
• Evolving neurologic examination.
• MRI
• Elevated temps in control group

Each point decrease in the Apgar score associated with 45% increase in odds of death or disability.

In multivariable analysis, Hypothermia treatment group lowered odds of the primary outcome (OR: 0.44) and death alone (OR: 0.5).

So, even though cooling helped, among the cooled, low 10 minute Apgar still increases risk of poor outcome

*Note: 1/5th of the children with 10-minute Apgar of 0 survived without disability to school age.


Evolving Exam

• Infants from NICHD study
• Assess exam at 6 hours (enrollment) and 72 hours and discharge and association with outcome

Changing Exam

- Increased risk of death/disability associated:
  - If no improvement in stage of HIE within the study intervention (72 hours), then significantly higher odds of death/disability than those who improved within the first 24 hours (P < .001).

<table>
<thead>
<tr>
<th>Time before any improvement in HIE stage</th>
<th>odds ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 72 hours</td>
<td>18.61</td>
<td>5.37-64.49</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>48-72 hours</td>
<td>0.74</td>
<td>0.17-3.21</td>
<td>.681</td>
</tr>
<tr>
<td>24 hours</td>
<td>REF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NICHD NRN: MRI, HIE and Cooling

• NICHD Hypothermia Study
• MRI score developed
  – Central reader
  – Based on injury location and characteristics
  – T1/T2, not diffusion

• 136 of 208 infants had MRIs
  – 73 in the hypothermia
  – 63 in the control group

Table 4  Relationship between the NICHD MRI pattern of injury and primary outcome within the two groups

<table>
<thead>
<tr>
<th>NICHD pattern of injury</th>
<th>Hypothermia group, n=73</th>
<th>Control group, n=63</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Death or disability</td>
<td>0 (7.9)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td></td>
<td>1A(0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>1B(0.0)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td></td>
<td>2A(20.0)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td></td>
<td>2B (69.6)</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td></td>
<td>3 (100.0)</td>
<td>6 (100.0)</td>
</tr>
</tbody>
</table>

Death p=0.37  N=19/73  N=27/63

|                        | p=0.10                  |
| Death                  | 0 (5.3)                 |
|                        | 0 (0.0)                 |
|                        | 0 (0.0)                 |
|                        | 1 (33.3)                |
|                        | 5 (21.7)                |
|                        | 2 (100.0)               |

Rate of disability p<0.0001  p<0.0001

|                        | 0 (2.8)                 |
|                        | 1 (10.0)                |
|                        | 0 (0.0)                 |
|                        | 1 (33.3)                |
|                        | 6 (33.3)                |
|                        | 11 (61.1)               |
|                        | 2 (100.0)               |

The two infants with normal MRI examination died after neonatal intensive care unit discharge: one infant died with viral pneumonia and the cause of death is missing for the other infant.

NICHD, National Institute of Child Health and Human Development.

Abnormal MRI's were decreased among cooled infants

Bad MRI was just as predictive of bad outcome among cooled and Controls.

Of note, The average age (mean±SD) when scans were obtained was 15±12 days. 39 infants had MRI scans obtained < 7 postnatal days e.


Similar in TOBY and ICE:

Childhood Outcomes: NRN Hypothermia

- N = 190 study participants w/ known outcomes 6 to 7 years
- Cognitive, attention and executive, visuospatial function, neurologic outcomes, physical and psychosocial health
- Primary Outcome: death or IQ < 70

## Childhood Outcomes: NRN Hypothermia

<table>
<thead>
<tr>
<th>Outcome2</th>
<th>Hypothermia (N = 97)</th>
<th>Normothermia (N = 93)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or IQ &lt; 70</td>
<td>46/97 (47%)</td>
<td>58/93 (62%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Death</td>
<td>27/97 (28%)</td>
<td>41/93 (44%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Death or severe disability</td>
<td>38/97 (41%)</td>
<td>53/93 (60%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean IQ scores</td>
<td>82</td>
<td>75</td>
<td>0.22</td>
</tr>
<tr>
<td>Moderate or severe disability</td>
<td>24/69 (35%)</td>
<td>19/50 (38%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Attention/exec dysfunction</td>
<td>2/48 (4%)</td>
<td>4/32 (13%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Visuospatial dysfunction</td>
<td>2/53 (4%)</td>
<td>1/36 (3%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

aRR, severity of HIE: 0.84 (95% CI 0.66 - 1.07)

Among Survivors....

Limitations of the pivotal Hypothermia Trials

• **Entry Criteria variation**
  - NRN, ICE: exam plus events/labs
  - TOBY, CoolCap: aEEG plus events/labs

• **Control Core temp of Control group variations**
  - CoolCap and NRN: no effort to control *core* temp among controls
  - TOBY: servo-controlled according to the abdominal skin temperature to maintain the rectal temperature at 37.0±0.2°C
  - ICE: controlled rectal temp to 37°C
  - nEURO: normal body temperature (ie, rectal temperature of 37°C [range: 36.5–37.5°C]) was maintained.

• **Sedation variations**
  - NRN: avoided sedation
  - TOBY: All infants underwent sedation with morphine infusions or with chloral hydrate *if they appeared to be distressed*
  - ICE: not established
  - nEURO: regular doses or equivalent infusions of morphine 0.1/kg Q4 hours or equivalent fentanyl
Optimizing Hypothermia
S Shankaran, PI, NICHD Study

- Is cooler/longer better than $33.5^\circ \times 72$ hours?
- Goal to enroll $> 700+$ kids

**Figure 2: Design outline of proposed trial**

<table>
<thead>
<tr>
<th>Duration of Cooling</th>
<th>Depth of Cooling</th>
<th>Margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 hours (Group X)</td>
<td>$33.5^\circ$ (Group A)</td>
<td>AX</td>
</tr>
<tr>
<td>120 hours (Group Y)</td>
<td>$32.0^\circ$ (Group B)</td>
<td>BY</td>
</tr>
<tr>
<td>Margin</td>
<td>X</td>
<td>Y</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Margin</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
</table>

Optimizing Cooling NICHD NRN

• Closed to accrual for safety and futility 11/27/2013
• 364 of planned 726 enrolled and randomized
# NICHD NRN Optimizing Cooling Results--Mortality

<table>
<thead>
<tr>
<th>Duration of cooling</th>
<th>Depth of cooling 33.5°C</th>
<th>Margin Duration 11% (72 h)</th>
<th>Depth of cooling 32.0°C</th>
<th>Margin Duration 16% (120 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 hours</td>
<td>7%</td>
<td>14%</td>
<td>16%</td>
<td>17%</td>
</tr>
<tr>
<td>120 hours</td>
<td>16%</td>
<td>17%</td>
<td></td>
<td>16%</td>
</tr>
</tbody>
</table>

*Margin Depth of cooling* 12% (std) 16% (exp)

- After adjustment for center and severity of encephalopathy:
  - Odds ratio in hospital mortality 120 vs. 72 hr: 1.74 (0.88 – 3.46)
  - Odds ratio in hospital mortality 32.0°C vs 33.5°C: 1.34 (0.68 – 2.67)
  - Interaction between duration and depth, p = 0.20

- Futility analysis: chances of seeing treatment benefit for longer or deeper < 2%

### Primary Outcome: \( 72\, h \) vs. \( 120\, h \)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>( 72, h )</th>
<th>( 120, h )</th>
<th>Adj RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Death or Moderate/severe disability</strong></td>
<td>56/176 (32%)</td>
<td>54/171 (32%)</td>
<td>0.92 0.68-1.25</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>23/176 (13%)</td>
<td>33/171 (19%)</td>
<td>1.39 1.02-1.89</td>
</tr>
<tr>
<td><strong>Moderate/severe disability</strong></td>
<td>33/153 (22%)</td>
<td>21/138 (15%)</td>
<td>0.68 0.41-1.11</td>
</tr>
<tr>
<td><strong>CP</strong></td>
<td>28/152 (18%)</td>
<td>18/138 (13%)</td>
<td>0.67 0.37-1.20</td>
</tr>
</tbody>
</table>

NICHD NRN Optimizing Cooling Study
Primary Outcome: 33.5°C vs. 32.0°C

<table>
<thead>
<tr>
<th></th>
<th>33.5°C</th>
<th>32.0°C</th>
<th>Adj RR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1º Outcome: Death or Moderate/severe disability</td>
<td>59/185 (32%)</td>
<td>51/162 (31%)</td>
<td>0.94 0.68-1.26</td>
</tr>
<tr>
<td>Death</td>
<td>26/185 (14%)</td>
<td>30/162 (19%)</td>
<td>1.17 0.67-2.04</td>
</tr>
<tr>
<td>Moderate/severe disability</td>
<td>33/159 (21%)</td>
<td>21/132 (16%)</td>
<td>0.71 0.36-1.39</td>
</tr>
<tr>
<td>CP</td>
<td>25/158 (16%)</td>
<td>21/132 (16%)</td>
<td>0.98 0.52-1.82</td>
</tr>
</tbody>
</table>

LATE HYPOTHERMIA
Evaluation of Systemic Hypothermia After 6 Hours of Age For Infants ≥ 36 wks with HIE: A Bayesian Evaluation

PI - A Laptook
Sub-committee: N Ambalavanan, E Bell, R Ehrenkranz, R Goldberg, S Shankaran, J Tyson, C Pedroza, A Hensman, K Osborne
NICHD: R Higgins
RTI: A Das

Laptook AR et al. Effect of Therapeutic Hypothermia Initiated After 6 Hours of Age on Death or Disability Among Newborns With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. JAMA. 2017 Oct 24;318(16):1550-1560.
Target Population

• Near term and term infants with HI and moderate/severe encephalopathy:
  • Arrive at a referral center after 6 hrs of age
  • Progress from stage I to II/III encephalopathy after 6hrs of age
  • Are not recognized to qualify until after 6hrs of age
  • Cooling cannot be initiated within 6 hrs of age (equipment/personnel availability)
### At Randomization

<table>
<thead>
<tr>
<th></th>
<th>Cooled (n=83)</th>
<th>Non-cooled (n=85)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age at randomization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 6 to ≤ 12 hr</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td>&gt; 12 to 24 hr</td>
<td>57</td>
<td>69</td>
</tr>
<tr>
<td><strong>Encephalopathy stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>73</td>
<td>88</td>
</tr>
<tr>
<td>Severe</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><strong>Other variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical seizures</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td>Anti-convulsants</td>
<td>56</td>
<td>75</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td>Blood transfusions</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Inotropic support</td>
<td>17</td>
<td>21</td>
</tr>
</tbody>
</table>
Primary and Secondary Outcomes: aRRs and Posterior Probability of Treatment Effect:

Table 3. Primary and Secondary Outcomes: aRRs and Posterior Probability of Treatment Effect

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. (%)</th>
<th>Enthusiastic Prior (RR, 0.72)</th>
<th>Neutral Prior (RR, 1.00)</th>
<th>Skeptical Prior (RR, 1.10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothermia (n = 78)</td>
<td>Noncooled (n = 79)</td>
<td>aRR (95% Credible Interval)</td>
<td>P-TB, %</td>
</tr>
<tr>
<td>Primary Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death or moderate-severe disability</td>
<td>19 (24.4)</td>
<td>22 (27.9)</td>
<td>0.78 (0.52-1.15)</td>
<td>90</td>
</tr>
<tr>
<td>Secondary Outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deathb</td>
<td>9 (11.5)</td>
<td>9 (11.4)</td>
<td>0.74 (0.45-1.21)</td>
<td>89</td>
</tr>
<tr>
<td>Moderate or severe disabilityc</td>
<td>10 (12.8)</td>
<td>13 (16.5)</td>
<td>0.74 (0.44-1.24)</td>
<td>87</td>
</tr>
<tr>
<td>Severe disabilityc</td>
<td>9 (11.5)</td>
<td>12 (15.2)</td>
<td>0.73 (0.43-1.23)</td>
<td>88</td>
</tr>
<tr>
<td>Moderate disabilityc,d</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild disabilityc</td>
<td>16 (20.5)</td>
<td>12 (15.2)</td>
<td>1.0 (0.62-1.62)</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations: aRR, adjusted risk ratio; P-TB, posterior probability of treatment benefit (risk ratio <1.0); RR, risk ratio.

Reference for the aRR is the noncooled group and the aRR is adjusted for level of encephalopathy (moderate, severe) and age at randomization (≤12 h, >12 h). Variables in the adjusted analyses were outcome, treatment, level of encephalopathy at randomization, and age at randomization.

Causes of death in the hypothermia group were asphyxia brain injury (n = 5), multiorgan failure (n = 1), persistent pulmonary hypertension (n = 1), respiratory failure associated with intractable seizures (n = 1), and intracranial hemorrhage (n = 1). Causes of death in the noncooled group were asphyxia brain injury (n = 5), multiorgan failure (n = 1), and meconium aspiration syndrome (n = 1), and 2 were without an assigned cause (after discharge).

Disability categories were defined as follows: severe included any of the following: a cognitive score less than 70, a Gross Motor Function Classification Score (GMFCS) level of 3 to 5, or blindness or hearing impairment with inability to follow commands despite amplification; moderate included a cognitive score between 70 and 84 and any of the following: a GMFCS level of 2, an active seizure disorder (antiepileptic drugs in use), or a hearing deficit with the ability to follow commands after amplification; and mild included a cognitive score between 70 and 84 or a cognitive score of 85 or greater with any of the following: a GMFCS level of 1 or 2, a seizure disorder (without medication), or a hearing deficit with the ability to follow commands without amplification.

Analyses could not be performed with 1 infant in each group.

From: Effect of Therapeutic Hypothermia Initiated After 6 Hours of Age on Death or Disability Among Newborns With Hypoxic-Ischemic EncephalopathyA Randomized Clinical Trial


So many words... translated, sort of...

76% posterior probability of reduced death or disability with hypothermia relative to the non-cooled group (adjusted posterior risk ratio, 0.86; 95% credible interval, 0.58-1.29).

The probability that death or disability in cooled infants was at least 1%, 2%, or 3% less than non-cooled infants was 71%, 64%, and 56%, respectively.
Conclusions

- There was a 3.5% reduction in death or disability (12.5% relative reduction) with cooling initiated at 6-24 hours of age.
- Bayesian analysis suggests possible treatment benefit:
  - 77% likelihood of reduced death or disability.
- Bayesian analysis is inconclusive for larger effect sizes:
  - 58% likelihood of more than a 10% reduction in death or disability.
- The results should not delay efforts to recognize HIE early and initiate hypothermia within 6 hrs of birth.
Hypoxic-Ischemic Brain Injury in the Term Neonate

Acute cell death-Continuum cell death: necrosis, apoptosis, autophagy

1° energy failure
- Cell-membrane effect

Secondary energy failure
- Nuclear/cell-programming effect

Excito-oxidative cascade
- Less excitatory neurotransmitters
- Less energy consumption
- Less caspase activation/apoptosis

Outcomes
- Depends on...
  - Intensity of insult
  - Brain maturity
  - Capacities for protein/RNA synthesis and DNA repair
  - Antioxidant status
  - Neurotropin requirements
  - Location in brain
  - Timing of therapeutic interventions

Adapted from D. Ferreiro

Ideals for Cooling **PLUS** Therapies

- Respond actively to environment/level of illness
- Contribute neuroprotective, neurotrophic factors
- Helpful w/ early *and* late response

From Bull Durham: “This is a simple game: You throw the ball, you hit the ball, you catch the ball.”
Hypoxia-ischemia

Primary cell death

Cell swelling

Connexin hemichannel opening

NMDA receptor hyperexcitability

Mitochondrial collapse

Excitotoxicity

Oxidative stress

Reperfusion

Primary injury

Latent phase ~ 6 hours

Pro-apoptotic signals

Epileptiform transients

Inflammation

Seizures

Secondary phase 6 to 72 hours

Cell swelling

Cell death

Impaired connectivity

Impaired maturation

Brain injury

Tertiary phase Days to months

Loss of trophic support

Chronic inflammation

COOLING PLUS?

Hypothermia Current Management PLUS
- Xenon
- Allopurinol
- Epo/Darbe
- Cells?

Cooling plus: *enrolling studies*

- Melatonin (NCT02621944, phase I and NCT03806816)
- Caffeine (NCT03913221, phase 1)
- Allopurinol (NCT03162653, phase III)
- Erythropoietin (NCT03079167 phase III; NCT01913340 phase III)
- Inhaled xenon (NCT02071394; phase II)
- Topiramate (NCT01765218; phase II)
- Sildenafil (NCT02812433, phase 1).
- **Cells** *(Duke studies ph 1 and II, NCT02551003, phase I; NCT02256618 phase I).*
Erythropoietin Neuroprotection

- EPO plus its receptor are expressed throughout the brain, in all cell types (neurons, astrocytes, oligodendrocytes, endothelial cells, microglia)
- EPO Receptors found throughout developing brain
- In vitro, EPO stimulates maturation of neurons, oligos and astrocytes
- Animal Knock-out models, without CNS EPO receptors, undergo massive early apoptosis
- EPO and receptor are induced by hypoxia

EPO Phase II trial

Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes, “NEATO”

• N = 50

• Intervention:
  – EPO (1000 U/kg IV; n = 24) or placebo
  – Doses: 1, 2, 3, 5, 7 postnatal days; first within first 24 postnatal hours

• Inclusion:
  – Moderate/severe HIE; perinatal depression (6 aspects), 5 min Apgar < 5, pH < 7.00 or base deficit ≥15, or resusc x 10 minutes
  – Cooled beginning in first 6 postnatal hours
  – MRI as “part of routine clinical care at 4 to 7 days of age.”

• Primary Outcome
  – AIMS Alberta Infant Motor Scale (12 months)

**EPO Phase II trial**
**Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes, or “NEATO” RESULTS**

<table>
<thead>
<tr>
<th>MRI (avg at 5.1 days, SD 2.3) at least 3 doses of Study Drug</th>
<th>EPO; n = 24</th>
<th>Placebo; N = 26</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global Brain Injury Score* (median; IQR) injury severity in 8 brain regions each hemisphere (0 – 3)</td>
<td>2; 0 - 9</td>
<td>11; 4 - 18</td>
<td>.01</td>
</tr>
<tr>
<td>Moderate/Severe Brain Injury (by MRI)</td>
<td>4%</td>
<td>44%</td>
<td>.002</td>
</tr>
</tbody>
</table>

**NEURODEVELOPMENT (12 months)**

<table>
<thead>
<tr>
<th>Alberta Infant Motor Scale</th>
<th>EPO; n = 24</th>
<th>Placebo; N = 26</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>53.2</td>
<td>42.8</td>
<td></td>
<td>.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Warner Initial Developmental Evaluation</th>
<th>EPO; n = 24</th>
<th>Placebo; N = 26</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.6</td>
<td>23.8</td>
<td></td>
<td>.05</td>
</tr>
</tbody>
</table>


Cord Blood Cells: Key Discoveries

• 1982: Cord blood includes hematopoetic progenitors (Nakahata)
• 1989: Cord blood provides progenitor and stem cells (Broxmeyer)
  – Before and after freezing/thawing
  – Hematopoetic-endothelial progenitors (CD34+)
• 1989 First reported cord blood allogeneic transplant: (Auerbach-Broxmeyer-Douglas-Gluckman-Kurtzberg)- 5 year old boy, Matthew Farrow from Salisbury, North Carolina with Fanconi’s and unaffected newborn sibling.
• 2001 Neural cell potential
  – Neural and astrocyte markers expressed by thawed nucleated HUCB cells (Sanchez-Ramos)
  – CD34 and other cells differentiated to neuronal, oligodendroglial, and astrocyte traits (Buzanska 2002)

• Nakahata T, Ogawa M. JCI 1982; 70:1324-8
• Gluckman E, et al NEJM; 321:1174-8
• Domanska-Januck K et al. *Int. J. Dev. Biol.* 2008;52:237-248 (review)
Therapeutic neovascularization, due to CD34+ cell transplantation (i.v.) after stroke, enhances neurogenesis, reduces apoptosis.

Adult animal model of stroke
C: CD34-; limited migration of neural stem cells into ischemic zone

F. CD34+ cell treated animal

TUNEL+ cells/HPF in CD34- (G) vs. CD34+ (H)

CD34 – Thin layer of neural progenitor cells
CD34+ Many more NPCs w/ CD34+


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Summary of Animal Work: an optimist’s view

- Ischemic brain injury
  - Perinatal hypoxia-ischemia
  - Transplantation of hUCB cells
  - Excitotoxic neuronal cell death
  - Apoptosis
  - Oedema
  - Blood brain barrier disruption
  - Inflammation
  - Astrocytic activation

Impaired functional outcome

- Recovery of motor and cognitive functions
  - Cell replacement (?)
  - Suppression of astroglial cell activation
  - Reduction of lymphocyte, granulocyte, and monocyte infiltration
  - Stimulation of endogenous neurogenesis / neuronal survival
  - Reduction of activated microglia
  - Decreased expression of pro-inflammatory cytokines
  - Expression of neurotrophic factors
  - Neovascularization and angiogenesis

Restored neural processing in the primary somatosensory cortex

Some evidence for all, but NOT cell replacement

Cells + Cooling

• P7 rats
• Unilateral carotid ligation and 2h @ FiO2 0.08.
• MRI within 2 hrs; ≥ 50% infarct
• Randomized to one of 4 groups,
• Interventions 6 hours post-injury
  – mesenchymal stromal cells derived from hUC Blood
    • Ipsilateral intraventricular injection
    • 1 x 10^5 cells
  – followed immediately by cooling

Cells + Cooling

Results

• Reduction in intact brain volume observed in HNC rats (red-injured controls)

• Treated:
  – HNM (blue: cells only)
  – HHM (green: cooled + MSCs)
  – HHC (orange: cooled only)

Autologous cord blood cells for brain injury in term newborns

Phase I open-label, single site, feasibility and safety study
NCT00593242
IND 14753
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
## Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cell recipients mean (min/max) or N (%)</th>
<th>N = 51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age, wks</td>
<td>39 (34/41)</td>
<td></td>
</tr>
<tr>
<td>Birthweight, kg</td>
<td>3330 (2120/4660)</td>
<td></td>
</tr>
<tr>
<td>SGA</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>LGA</td>
<td>6 (12)</td>
<td></td>
</tr>
<tr>
<td>C-section delivery</td>
<td>35 (78)</td>
<td></td>
</tr>
<tr>
<td><strong>Outborn</strong></td>
<td><strong>16 (31)</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>23 (45)</td>
<td></td>
</tr>
</tbody>
</table>
### Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cell recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (min/max) or N (% or IQR)</td>
</tr>
<tr>
<td></td>
<td>N = 51</td>
</tr>
<tr>
<td>5 minute Apgar &lt; 5</td>
<td>42 (82)</td>
</tr>
<tr>
<td>10 minute Apgar &lt; 5</td>
<td>27 (49)</td>
</tr>
<tr>
<td><strong>Cord pH</strong></td>
<td><strong>6.97 (6.86/7.1)</strong></td>
</tr>
<tr>
<td>Cord pH &lt; 7</td>
<td>28 (56)</td>
</tr>
<tr>
<td>Base deficit ≥ 16</td>
<td>27 (of 37; 67)</td>
</tr>
<tr>
<td>NICHD score*</td>
<td>24 (19/29)</td>
</tr>
<tr>
<td>NICHD score* &gt; 30</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Seizures</td>
<td>19 (37)</td>
</tr>
</tbody>
</table>

# Feasibility Measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean or N (range or %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume Collected, ml</td>
<td>35 (3, 178)</td>
</tr>
<tr>
<td># Cells post processing (x 10^8)</td>
<td>4.4 (0.77, 35)</td>
</tr>
<tr>
<td>Time to first infusion</td>
<td></td>
</tr>
<tr>
<td>&lt; 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 (22)</td>
</tr>
<tr>
<td>3*</td>
<td>3 (6)</td>
</tr>
<tr>
<td>2</td>
<td>24 (47)</td>
</tr>
<tr>
<td>1</td>
<td>12 (24)</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>Outcome</th>
<th>Cell recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 51 (%)</td>
</tr>
<tr>
<td>ECMO</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Deaths (deaths were post-discharge, but sent home with palliative care plan)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Seizure meds at discharge</td>
<td>11 (22)</td>
</tr>
<tr>
<td>100% Oral feeds at discharge</td>
<td>40 (80)</td>
</tr>
</tbody>
</table>
Survival with 1 yr Bayley III scores $\geq 85$ in 3 domains

<table>
<thead>
<tr>
<th>Survival with all 3 Bayley domain scores $\geq 85$</th>
<th>25 (64%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival to one year</td>
<td>37 (95%)</td>
</tr>
<tr>
<td>Bayley III scores (median, IQR)</td>
<td></td>
</tr>
<tr>
<td>Cognitive</td>
<td>100 (90 – 105)</td>
</tr>
<tr>
<td>Motor</td>
<td>94 (70 – 100)</td>
</tr>
<tr>
<td>Language</td>
<td>94 (86 – 100)</td>
</tr>
</tbody>
</table>

* 2 cell recipients died after discharge
What did we learn?

- Key connections: Neonatologists with Obstetricians and Lab Processors and Follow-up *(and families!)*
- With most babies outborn, collections can be a challenge
- A dose can be obtained from a very small blood volume
A Phase II Multi-site Study of Autologous Cord Blood Cells for Hypoxic Ischemic Encephalopathy (HIE) IND 14753 (BABYBAC II)

C. MICHAEL COTTEN MD MHS
JOANNE KURTZBERG MD
Randomization

- After informed consent, randomization to placebo or study product.
- NICU study team collects demographic info and severity info.
- Randomization:
  - Web-based
  - 24/7 availability by DCC
  - Only unblinded laboratory personnel will be trained on randomization and have knowledge of study arm.
- Randomization stratified by severity of encephalopathy
- Randomized to:
  - Study product: $2 - 5 \times 10^7$ cells/kg/dose
  - Placebo (diluted whole cord blood)
STUDY STATUS – BABYBAC II

Duke University – 18 SUBJECTS ENROLLED
UF Gainesville – 3 SUBJECTS ENROLLED
UF Jacksonville- 3 SUBJECTS ENROLLED
CHOP- 1 SUBJECT ENROLLED
WSU- 3 SUBJECTS ENROLLED
MGH and BWH- 5 SUBJECTS ENROLLED
UAB – 3 SUBJECTS ENROLLED

4 sites screened, but no enrollment

• 36 total enrolled
• Study funding stopped
• Follow-up underway
• Regulatory and logistic challenges
hCT-MSC's for Infants with HIE

C. Michael Cotten MD MHS
Professor of Pediatrics/ Division of Neonatal-Perinatal Medicine
Joanne Kurtzberg MD
Jerome S. Harris Professor of Pediatrics/ Division of Blood and Marrow Transplantation/ Marcus Center for Cellular Cures

IRB Review NOV 2018
Approach

• Open-label, phase I, dose escalation trial of allogeneic hCT-MSC’s in newborn infants with moderate to severe HIE and treated with TH.

• **Population:** n = 6; ≥ 36 weeks gestation; autologous cells NOT available.
  - (≈ 30 infants treated w/ TH annually at Duke; 1/3rd w/ autologous cells collected.)

• **Intervention:** hCT-MSCs: 2x10⁶ cells/kg per intravenous infusion;
  - hCT-MSCs manufactured at GMP facility at Duke (2nd passage cells)
  - 1st 3 subjects one infusion during TH
  - 2nd 3 subjects add 2nd infusion 2 months post first.

• **Comparison:** Exploratory comparisons with infants who received TH but no cells, and with autologous cell recipients from our prior phase I study.

• **Outcome(s):** Primary focus on safety outcomes:
  - Infusion reactions, infections and significant adverse events that are, or could be, related to the study intervention.
  - Assessments at time of infusion, 24 hours after infusion, and 7-10 days after infusion.
  - Chimerism to be assessed 24 hours, 10 days and 2 months after infusions.

• **6 enrolled July 2019. 6th baby got 2nd infusion**
What we didn’t discuss

• What about preemies? *NRN Clinical trial underway*

• Seizures/EEG monitoring: seizure burden (subclinical included) associated with worse outcome, but what’s best treatment for seizures?

• Analgesia during cooling: practice variations, what’s the best approach?

• Identifying community referrals: who to send? When to decide?

Berube M, et al PAS2017
Thank You

- Colleagues at Duke
  - Ron Goldberg, Joanne Kurtzberg, Kim Fisher, NPRU
  - Chad Grotegut, Geeta Swamy, Amy Murtha, MFM and L and D
  - Barb Waters-Pick, Cell lab
  - Monica Lemmon, Carolyn Pizoli
  - William Malcolm, Ricki Goldstein
  - Nursing colleagues!! NPRU!!!
- Colleagues at the NICHD NRN:
  - Seetha Shankaran, Abbot Laptook
- Colleagues at the BABYBAC II sites
  - Sara Bates, Terrie Inder, Mohamed El-Dib, Michael Weiss
- Colleagues worldwide:
  - Won Soon Park, Mako Nabetani, Mario Ruediger
- Most of all: babies and their families!!
Considerations for Phase II

- Consistent cooling approach
- OB-MFM champion
- Ready to transport cells

<table>
<thead>
<tr>
<th>Seizures</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of Consciousness</td>
<td>Level of Consciousness 25363</td>
</tr>
<tr>
<td>Spontaneous Activity</td>
<td>Spontaneous Activity 25364</td>
</tr>
<tr>
<td>Posture</td>
<td>Posture 25365</td>
</tr>
<tr>
<td>Tone</td>
<td>Tone 25366</td>
</tr>
<tr>
<td>Primitive Reflexes</td>
<td>Primitive Reflexes 25373</td>
</tr>
<tr>
<td>Autonomic System</td>
<td>Autonomic System 25389</td>
</tr>
</tbody>
</table>

I personally reviewed the history and performed the physical exam at 3:10 PM on 11/06/2014.
Systemic Hypothermia in Neonates With Hypoxic-Ischemic Encephalopathy (HIE)

1. Patients with a presumptive diagnosis of hypoxic-ischemic encephalopathy who meet ALL of the following five criteria are eligible for this order set. Check off each positive finding:
   1. Gestational Age greater than or equal to 35 weeks gestation
   2. Birth weight greater than or equal to 1.8 kg
   3. less than or equal to 6 hours since insult occurred
   4. ONE OR MORE of the following predictors of severe HIE:
      a) pH less than or equal to 7.0 with base deficit of greater than or equal to 16 on arterial blood gas determination (base excess more negative than -16)
      b) pH 7.01-7.15, base deficit 10-15.9 or no blood gas available and acute perinatal event (cord prolapse, heart rate decelerations, uterine rupture) and either APGAR less than or equal to 5 at 10 minutes or assisted ventilation at birth required for greater than or equal to 10 min
   5. Seizures or 3 of 6 of the following:
      | Clinical Criteria | Signs of Encephalopathy |
      |-------------------|-------------------------|
      |                   | Moderate Encephalopathy | Severe Encephalopathy |
      | 1. Level of consciousness | Lethargic | Stupor/coma |
      | 2. Spontaneous activity | Decreased activity | No activity |
      | 3. Posture | Instal fission, complete extension, frog leg posture | Decerebrate |
      | 4. Tone | Hypotonia (focal or general), hypertonia (focal or truncal) | Flaccid |
      | 5. Primitive reflexes | Suck | Weak or bite | Absent |
      |                   | Moro | Incomplete | Absent |
      | 6. Autonomic system | Pupils | Constricted | Skew deviation/dilated/non-reactive to light |
      |                   | Heart rate | Bradycardia | Variable |
      |                   | Respirations | Periodic | Apnea or intubated |

Exclusion Criteria
- Presence of lethal chromosomal abnormalities
- Severe IUGR
- Significant intracranial hemorrhage with a large intracranial hemorrhage (Grade III or intraparenchymal echodensity (Grade IV))(Note: may start hypothermia without obtaining HUS if not immediately available. Should be obtained as soon as possible after the start of hypothermia.)

11 NICUs statewide

http://hopefn3.org/members/protocols-and-guidelines/
Florida Neonatal Neurologic Network
Mike Weiss: U Florida
DCC and infant’s blood volume

Still end w/ 13.8 mL/kg placental volume
After 3 minutes (in healthy cord+placenta)

Inclusion Criteria

- Is cord blood available?
- Moderate/Severe HIE Per NICHD NRN Hypothermia Trials
  - Assess history and blood gasses
  - Assess neuro-exam in first 6 postnatal hours
Exclusion Criteria

- Major congenital or chromosomal abnormalities
- Severe growth restriction (birth weight <1800 g)
- Opinion by attending neonatologist that the study may interfere with treatment or safety of subject
- Moribund neonates for whom no further treatment is planned
- Infants born to mothers known to be HIV, Hepatitis B, Hepatitis C or who have active syphilis or CMV infection in pregnancy
- Infants suspected of overwhelming sepsis
- ECMO initiated or likely in the first 48 hours of life
- ALL blood gases (cord and postnatal) done within the first 60 minutes had a pH >7.15 AND a base deficit < 10 mEq/L (source can be arterial, venous or capillary)
- ***ADDED CELL CRITERIA: positive gram stain
- Known Zika added
- Chorio added, defined per August 2017 guidance from ACOG Clinical Practice Committee.
  - maternal temperature is greater than or equal to 39.0°C or
  - maternal temperature is 38.0–38.9°C, which persists when repeated after 30 minutes, and one additional clinical risk factor present
    - (maternal leukocytosis,
    - purulent cervical drainage, or
    - fetal tachycardia).
1997 Neonatal Animal Models Summary

- **Post** H-I injury...
- Cooling during reperfusion
  - 2 - 6°C reduction; 3- 72 hours
  - 2 animal models (pigs and rats)
  - 6 experiments
  - 25 – 80% reduction in neural damage
- Mechanisms (by 1997)
  - Reduction in excitatory amino acids
  - Reduction in nitric oxide and oxygen free radicals
  - Reduction in apoptosis

Randomized Trial of Targeted Temperature Management with Whole Body Hypothermia for Moderate and Severe Encephalopathy in Premature Infants 33-35 Wks Gestation – A Bayesian Study...goal 168, now at ...

PI – R Faix

Sub-committee: A Laptook (Vice Chair), S Shankaran, B Yoder, J Beachy, P Sanchez, M Laughon, K Dysart, J Tyson, C Pedroza, R Heyne

A Hensman, D Vasil

NICHD: R Higgins

RTI: A Das, B Eggleston, M Crawford .....
Why didn’t cells + cooling work as well? Potential ‘pro-inflammatory’ mechanism

Co-culture MSCs with brain slices from injured animals either kept normothermic (NT) or hypothermic (HT)
- Co-culture x 48 hrs
- Dashed lines are sham control levels (mRNA; cytokine levels)
- Bars are ‘relative to sham controls’

- HIGHER LEVELS OF ‘BAD THINGS’ w/ HT (hypothermia-treated) cells
- LOWER LEVELS OF ‘GOOD THINGS’ w/ HT (hypothermia-treated cells)

## Morbidities During Hospitalization

<table>
<thead>
<tr>
<th></th>
<th>Cooled n=83</th>
<th>Non-cooled n=85</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>MAS</td>
<td>23</td>
<td>27.7</td>
<td>20</td>
</tr>
<tr>
<td>PPHN</td>
<td>20</td>
<td>24.1</td>
<td>13</td>
</tr>
<tr>
<td>iNO use</td>
<td>14</td>
<td>16.9</td>
<td>15</td>
</tr>
<tr>
<td>ECMO</td>
<td>3</td>
<td>3.6</td>
<td>2</td>
</tr>
<tr>
<td>Cardiac ischemia</td>
<td>14</td>
<td>16.9</td>
<td>13</td>
</tr>
<tr>
<td>Hypotension + pressor</td>
<td>28</td>
<td>33.7</td>
<td>24</td>
</tr>
<tr>
<td>Oliguria</td>
<td>19</td>
<td>22.9</td>
<td>18</td>
</tr>
<tr>
<td>Hepatic dysfunction</td>
<td>19</td>
<td>22.9</td>
<td>24</td>
</tr>
<tr>
<td><strong>DIC</strong></td>
<td>9</td>
<td>10.8</td>
<td>4</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>2</td>
<td>2.4</td>
<td>1</td>
</tr>
<tr>
<td>DNR + withdraw support</td>
<td>6</td>
<td>7.2</td>
<td>8</td>
</tr>
<tr>
<td>DC-GT of gavage</td>
<td>1/74</td>
<td>1.4</td>
<td>1/78</td>
</tr>
</tbody>
</table>