Advancing Therapies for Neonatal Brain Injury

Neonatal Brain Injury Laboratory:
Drs. Martin, Zhang, Chavez-Valdez, Burnsed
Neurosciences Intensive Care Nursery Program:
Drs. Hartman, Graham, Everett, Poretti, Tekes, Huisman, Lee, Kannan, Fatemi, and Johnston

Presented by: Frances J. Northington, MD
February 9, 2015

Advancing Therapy for Neonatal Brain Injury

Additional Slides and Data from:
Sandra Juul
Dept. of Pediatrics, University of Washington, Seattle, WA
Cobi J. Heijnen
Laboratory of Neuroimmunology, MD Anderson Cancer Center, Houston, TX
Sujatha Kannan, Elizabeth Nance and Rangaramanujam Kannan
Depts of Anesthesiology, CCM and Ophthalmology, JHU, Baltimore, MD
Dan Wu and Jiangyang Zhang
Depts of Biomedical Engineering and Radiology, JHU Baltimore, MD
Damjan Oskredkar and Marianne Thoresen
University of Oslo, Oslo, Norway

Presented by: Frances J. Northington, MD
February 9, 2015

Conflict of interest:

The author receives grant funding for Autoregulation research from Covidien.

Funding:

AG016282- (LJM), HD070996- (FJN), March of Dimes, Coviden (Lee –PI), NINDS- (Zhang PI)
Disclosures

• Erythropoietin is not labeled for use as a neuroprotective treatment.
• Cooling Cure device is not an approved device and is not being used in human studies.

Advancing Therapy for Neonatal Brain Injury

• Clinical studies – Technology for Low resource settings.
• New and Upcoming Therapeutic Trials
• Biomarkers of Neonatal Brain Injury
• Novel Science in NBI: Advanced image analysis
  Dendrimer delivery of drugs to the brain
• Opportunities for individualized medicine
  Importance of rewarming period.
• Hypothermia Caveats
  Gender and Inflammation

Epidemiology of Neonatal Encephalopathy

Higher Resource Countries
• NE- 3-4/1000
• HIE- 1.5-2/1000
• Perinatal stroke-1/1500-1/6000

International Data
• HIE- 23% of all neonatal deaths
• Top 20 leading causes of burden of disease across the lifespan
• 5th –cause of death in children <5
Global Mortality Statistics:
~4 million neonates die per year

Source: Lawn et al, Lancet 2005 Slide courtesy of S. Juul, MD, UW

---

Therapeutic Hypothermia for Newborns with HIE in the Developing World

Design a cost-effective hypothermia device that requires less training and electricity in order to reduce the mortality and disability rates caused by HIE in the developing world.
Efficiently Reaches target temperature

Time Took to Reach 33.5°C: ~1 hr 40 min
Matches with our modeling!

Cost Comparison

<table>
<thead>
<tr>
<th>Component</th>
<th>Cost</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Ceramic Pots</td>
<td>$14.00</td>
<td>Akron Mils</td>
</tr>
<tr>
<td>Sand</td>
<td>$0.61</td>
<td>Logistic Chemisols</td>
</tr>
<tr>
<td>Scissor Stand</td>
<td>$10</td>
<td>Brilife (Shanghai) International Trading Co., Ltd.</td>
</tr>
<tr>
<td>Microprocessor with analog to digital convertor</td>
<td>$10.39</td>
<td>Shenzhen Shijibaike Electronics Co., Ltd.</td>
</tr>
<tr>
<td>Circuit board</td>
<td>$0.50</td>
<td>Shenzhen Hongmy Precision Circuit Co., Ltd.</td>
</tr>
<tr>
<td>Medical Grade Thermisters</td>
<td>$2.00</td>
<td>Zhaoqing Exsense Electronics Technology Co., Ltd.</td>
</tr>
<tr>
<td>Cotton Blanket</td>
<td>$1.40</td>
<td>Shanghai Baby Products</td>
</tr>
<tr>
<td>5 Hours of Semi-skilled Labor</td>
<td>$0.45</td>
<td>Pooja Group</td>
</tr>
<tr>
<td>Cotton Fabric</td>
<td>$1.97</td>
<td>Crafty Cuts</td>
</tr>
<tr>
<td>LLDPE coating</td>
<td>$0.03</td>
<td>Fortune Plastics</td>
</tr>
<tr>
<td>Teflon Coating</td>
<td>$0.25</td>
<td>Suzhou Brisun Trading Co.</td>
</tr>
<tr>
<td>Total Cost of Device</td>
<td>$41.60</td>
<td></td>
</tr>
</tbody>
</table>

99% Saving

$12,000

U.S. Mortality Statistics:
~ 4 million are born per year

Centers for Disease Control and Prevention,
Slide courtesy of S. Juul, MD, UW
Neurodevelopmental Costs of Prematurity in U.S.

- Prematurity: 11.7% in 2011 (down from 12.8% in 2006)
  - 1/3 infant deaths in U.S.
  - 45% of children with cerebral palsy (CP)
  - 35% of children with visual impairment
  - 25% of children with cognitive or hearing impairment
- Care of preterm infants accounts for more than half of pediatric health care dollars spent
- Lifetime cost per patient with CP in the US was estimated at nearly $1 million in 2003

Slide courtesy of S. Juul, MD, UW

Neurodevelopmental Outcomes in Term Infants in U. S.

- Hypoxic ischemic encephalopathy - 1.7/1000 live births
  - 7,000/year in US, 50% death or severe NDI= 3500
- Pulmonary hypertension – 1 to 2/1000 live births
  - 6,000/year in US, 25% NDI, neurosensory hearing loss= 1500
- Cyanotic heart disease – 1.4/1000 live births
  - 5,600/year in US, 50% NDI= 2800
- Stroke – 1/4000 live births
  - 1000/year in US... variable outcomes~ 500
- Non accidental trauma, other trauma

Slide courtesy of S. Juul, MD, UW

Hypothermia is incompletely neuroprotective at 18-24 m: Major Clinical Trial Results

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Coolcap</th>
<th>TOBY</th>
<th>NICHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or severe disability</td>
<td>66% v. 55%</td>
<td>53% v 45%</td>
<td>62% vs. 44% p</td>
</tr>
<tr>
<td></td>
<td>p=0.1, 0.04</td>
<td>p=0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADJUSTED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disabling CP</td>
<td>31% v. 19%</td>
<td>41% v. 28%</td>
<td>30% vs. 19%  p=0.20</td>
</tr>
<tr>
<td></td>
<td>p=0.12</td>
<td>p=0.03</td>
<td></td>
</tr>
<tr>
<td>Survival without neurological abn</td>
<td>28 % v. 44%</td>
<td>28 % v. 44%</td>
<td>28 % v. 44%</td>
</tr>
</tbody>
</table>

Adapted from D. Ferriero

2/9/2015
### Hypothermia is incompletely neuroprotective - 6-7 Year Outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hypothermia</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death or IQ&lt;70</td>
<td>47%</td>
<td>62%</td>
<td>0.06</td>
</tr>
<tr>
<td>Death</td>
<td>28%</td>
<td>44%</td>
<td>0.04</td>
</tr>
<tr>
<td>Death/Mod/Sev</td>
<td>53%</td>
<td>65%</td>
<td>0.14</td>
</tr>
<tr>
<td>Death or Sev</td>
<td>41%</td>
<td>60%</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Shankaran et al
Volume 366(22):2085-2092
May 31, 2012

### JHU NICN

**Infants treated Hypothermia**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>141</td>
<td>110</td>
</tr>
<tr>
<td>Inborn</td>
<td>10 (8-33)</td>
<td>100</td>
</tr>
<tr>
<td>Outborn</td>
<td>18 (6-69)</td>
<td>88</td>
</tr>
<tr>
<td>LOS Median (Range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding Outcome at Discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Step Down</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Still in NICU</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Home Disposition</td>
<td>31%</td>
<td></td>
</tr>
</tbody>
</table>

### Hypothermia is not Neuroprotective after Infection-Sensitized Neonatal Hypoxic-Ischemic Brain Injury

Damjan Osredkar
Marianne Thoresen
Hershey Conference
June 3 – 6, 2014
Which additional Neuroprotective Agents are Ready for Bench to Bedside Translation

<table>
<thead>
<tr>
<th>Melatonin</th>
<th>Epo</th>
<th>NAC</th>
<th>Epo mimetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of Administration</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Starting dose</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Teratogenicity</td>
<td>10</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Benefit</td>
<td>8</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>FDA approved</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Total score</td>
<td>45</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>Rank</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>


Neuroprotection Contenders- based on presentations at 9th Hershey Conf.

- Erythropoietin (Epo)
- Melatonin
- Xenon, Argon?
- N-acetyl cysteine, Vitamin D
- Allopurinol
- Caffeine
- IGFl, EGF, VEGF
- Antiepileptics
- Hemichannel 43 blockade
- Na+/K+ ATPase
- Cell-based therapies

Current studies of Adjuvant Therapies for Hypothermia Therapy of Neonatal HI

Inhaled xenon and cooling (NCT01545271 and NCT00934700),
Safety of erythropoietin- NEAT O (NCT00719407), *
Darbepoetin and hypothermia (NCT0147105), *
Topiramate plus hypothermia (NCT01241019)- Late hypothermia NCT00614744
Hypothermia and Magnesium Sulphate NCT01646619
Safety of Clonidine during Therapeutic Hypothermia NCT01862250
Other Studies of Hypothermia Therapy of Neonatal Brain Injury

Late hypothermia NCT00614744
Optimizing cooling (NCT01192776)-not enrolling

Premie Hypothermia NCT01793129
Hypothermia In Hyperammonemia Encephalopathy NCT01624311
Hypothermia during ECMO NCT01675388

Hypothermia for NE in Low Resource Settings- NCT01760629

—

TH and Epo in Nonhuman Primate Model of HIE

Traudt et al. 2013. Dev Neurosci 35: 491-503

Slide courtesy of S. Juul, MD, UW

Phase I Trial of Neonatal Epo in Perinatal HIE (NEAT Trial)

Methods: Epo dose-escalation open-label study, N=24
All subjects met criteria for moderate HIE and were cooled
•250 (N=3),
•500 (N=6),
•1000 (N=7)
•2500 U/kg/dose (N=8)

Infants received up to 6 doses of Epo IV QOD starting at <24h of age
There were no safety issues identified

Wu et al. Pediatrics 2012;130:683-91

—
NEAT 1 Follow Up

- 24 infants were followed for 22 +/- 7.4 months.
- There were no deaths (6 expected).
- 1 child (4.5%) had a moderate to severe disability; this child had quadriplegic CP and GMFCS 3 (6 expected).
- MRI findings:
  - 11 (46%) had a normal brain MRI and a normal outcome.
  - 8 (34%) had moderate to severe brain injury on MRI, including the patient with moderate to severe disability.
  - 7 had moderate to severe watershed distribution injury and exhibited the following outcomes: normal (3), mild language delay (2), mild hemiplegic CP (1), and epilepsy (1).

Submitted for publication

Cognitive Outcomes of Preterm Infants Tx with Darbe, Epo, or Placebo

- Prospective, randomized, masked, multicenter study.
- 500 to 1250 gm.
- Study drug administered: 48 hours to 35 weeks PMA.
  - N=27 Darbe (10 microgram/kg, 1 x /week S.C.)
  - N=29 Epo (400 U/kg, 3 x/week subcutaneously)
  - N=24 Placebo (sham dosing 3x/week) given.
- Follow up: 18 to 22 months PMA.
  - Bayley Scales of Infant Development III.
  - Standardized neuro exam.


<table>
<thead>
<tr>
<th>Cognitive Outcomes of Preterm Infants Tx with Darbe, Epo, or Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayley Scales of Infant Development and Neurodevelopmental Outcomes</td>
</tr>
<tr>
<td>Darbe (N=27)</td>
</tr>
<tr>
<td>Composite cognitive score</td>
</tr>
<tr>
<td>NDI (3 (11.1%))</td>
</tr>
<tr>
<td>NDI or Death (4 (14.3%))</td>
</tr>
<tr>
<td>Cerebral Palsy (0)</td>
</tr>
</tbody>
</table>

Planned or Ongoing studies of Epo for term and preterm infants

**Term Neuroprotection**
- H Liley, Australia: PAEAN study (phase III funded)
  - N= 300, Epo 1000 U/kg; protocol matches NEAT
- J Patkai, France (phase III, enrolling)
  - N = 330, Epo 1000U/kg + HT
- A Pappas/S Shankaran: NICHD NRN
  - Phase II/III Epo + HT (proposed study—details unclear)
- Wu et al: HEAL U01 planned

**Preterm Neuroprotection**
- Swiss Trial – enrollment completed
- **PENUT trial 275 / 940 enrolled!**

---

**Serum cytokines in HIE: A biomarker of outcome**


---

**Effect of Hypothermia on Peripheral Immune Cells**

Astrocyte responses in PreClinical Models of HI


Hypothermic Regulation of the Astrocyte Proteome

Seo et al., Electrophoresis. 2012. 33;3835-3848

Table 2. Canonical pathway analysis of hypothermia-modulated proteins

<table>
<thead>
<tr>
<th>Pathway</th>
<th>P-value (-log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate receptor signaling</td>
<td>4.57</td>
</tr>
<tr>
<td>14-3-3-mediated signaling</td>
<td>2.35</td>
</tr>
<tr>
<td>Glutamate metabolism</td>
<td>2.20</td>
</tr>
<tr>
<td>Glutathione metabolism</td>
<td>1.96</td>
</tr>
<tr>
<td>Oxidative stress response</td>
<td>1.77</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>0.648</td>
</tr>
</tbody>
</table>
Progressive Predictive Value of Serum GFAP for abnormal early outcome following HI and Hypothermia - Graham & Everett

GFAP levels (determined by MS/MS) on cord/admission blood predictive

A threshold with 100% PPV identified

Marked spike in levels post cooling identifies risk period during rewarming phase

Predictive value of serum GFAP for PVWMI in Preterm Neonates

GFAP as a Marker for Periventricular White Matter Injury in Preterm Neonates

Stewart, Graham et al, AJOBGYN, 2013

Effect of rewarming on cell death following HI and Hypothermia

Unpublished data - Courtesy of Dr. J Lee Summers
Oscillating gradient diffusion MRI (OG-dMRI) improves tissue contrasts in neonatal brain.

\[ D_{cc} = 1.8 \, \mu m^2/ms \]
\[ D_{cyto} = 0.5 \, \mu m^2/ms \]
Regional differences in Injury Progression in Hippocampus defined by $\Delta f_{ADC}$

OG-dMRI offers additional information about microstructural change after Neonatal HI

Pseudo-normalization may relate to cell swelling / cell membrane permeability changes

Mild Injury- increased detection with Oscillating gradient diffusion

Wu and Zhang- unpublished data
Summary

- OG-dMRI offers additional information about microstructural change after HI
  - Pseudo-normalization seen in severe edema may relate to cell swelling / cell membrane permeability changes
  - Expanded lesion area around WM in type II injury may relate to astrocytes activation in these areas

Dendrimers: ‘Tree-like polymers’

- Dendrimers are well-defined, tree-like synthetic polymers, with a size of ~4 – 20 nm.
- Flexible, open structure.
- Each component of the tree can be manipulated.
- Biocompatible.
- Can be made biodegradable.
- Multifunction (therapy, imaging, targeting)

Improvement in Motor Function: DNAC tx

Dramatic improvement in motor function seen by Day 5, with dendrimer-NAC treatment

Kannan S et al, Science Translational Medicine, 2012
Myelination, Neuronal injury, Microglial Activation

- Associated with decrease in markers of oxidative injury
- Increase in glutathione levels
- Decrease in inflammation at day 5 of age
- Decrease in neuronal injury

Dose-response intranasal application of MSC on brain damage

- HI at day 9
- Intranasal MSC: 0.25; 0.5; 1 x 10^6 day 10 post HI
- Damage Day 28

Motor function: Cylinder rearing test

- HI at day 9
- Intranasal MSC: 0.25; 0.5; 1 x 10^6 day 10 post HI
- Sensorimotor function Day 28

Intranasal MSC treatment restores cognitive deficits

Training session
- Exposure to a new mouse
- Cognitive function: Social recognition task

Discrimination task
- 5 min or 3 h after training
- New vs. Now Familiar

Record preference for new over now familiar mouse

JHH- NICN
Infants treated with Hypothermia for HIE
Incidence and Outcome stratified by Gender

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Home</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Stepdown</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Deceased</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Length of Stay</td>
<td>5-31 days</td>
<td>1-55 days</td>
</tr>
</tbody>
</table>

Overall: 61% male; 39% female

Neonatal Mouse Model of HI and Hypothermia - J. Burnsed

Postnatal day 10
C57BL6 mice

Vannucci model:
- Unilateral carotid ligation +
- 45 min of hypoxia (0.08 FI02)

Hypothermia 31°C x 4hrs
Normothermia 36°C x 4hrs
Sex Differences in response to HI injury and treatment with TH

Burnsed, unpublished

Sex Differences in response to HI injury and treatment with TH

Early Biochemistry of Injury in Males
Synapse injury after HI, possible protection with Hypothermia in Males

Male HI/Hypothermia Synapse Western

Activation of ER stress in male mice following neonatal HI

Hypotheses:
- Neonatal HI induces early ER stress, activating the UPR differentially in males and females.
Conclusion: There is much more work to be done to protect the baby brain.

- Adjuvant and Low cost therapies
- Individualizing Intra-hypothermia care
- Supporting recovery and repair processes initiated by Glia
- Harnessing and Applying information provided by advanced imaging techniques
- Platforms for delivering drugs in a targeted, sustained manner for brain injury: implications in other neurodegenerative diseases
- Recognizing gender differences in response to injury and therapy