Epo--Hematological and Non-Hematological Effects

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University of Washington
Disclosures

- I have nothing to disclose
- Use of Epo for neuroprotection is off label
Objectives

Erythropoietin (Epo)

- Hematologic Effects
- Nonhematologic Effects
  - Preterm Brain Injury
  - Term Brain Injury (HIE)
Erythropoietin (Epo)

- Principle growth factor responsible for erythropoiesis
- 165-amino acid protein with post translational additions:
  - 3 N-linked carbohydrate chains, 1 O-linked carbohydrate chain
- Dosing 3 times a week sq or daily IV

Darbepoetin

- Synthetic erythropoietic molecule
- 165-amino acid protein with post translational additions:
  - 2 more N-linked chains and 8 more sialic acid residues
- Dosing once a week sq or iv
Erythropoietin (Epo)

• Epo does not cross the placenta
  – Fetal and maternal erythropoiesis occur independently
  – Factors responsible for fetal RBC production are produced by the fetus, and possibly the placenta

• Liver is the primary source of Epo in the fetus
  – Anephric fetuses have normal Epo levels and Hcts
  – At term, 75% of the circulating Epo is of hepatic origin

• Over time, primary site of synthesis is kidney
Neonatal Transfusion

- 80% of VLBW and 95% of ELBW infants are transfused
- 80% receive more than 1 transfusion
- Many are exposed to more than 1 donor
- Transfusions are associated with risk
  - Infection (viral, bacterial, prions)
  - Fluid overload, electrolyte disturbances
  - BPD, increased diuretic use
  - Immunomodulation
  - ROP, NEC?
  - Transfusion reactions, graft vs. host disease
Contributors to Anemia in Preterm Infants

• Iatrogenic
  – Phlebotomy loss may exceed circulating blood volume
  – Highest phlebotomy loss is in 1st 2 weeks of life
• Short RBC life span
• Production must keep up with growth (RBC/kg body mass)
• Inadequate iron stores
  – 80% of iron transfer to fetus occurs in 3rd trimester
  – Postnatally, poor enteral iron absorption
• Anemia of Prematurity
Phlebotomy loss

• 24-26 wks gestation - first 6 weeks of life
  – Mean: 81.8 mL ± 42.5 mL
  – Range: 34.6 to 191.2 mL

• < 28 wks gestation, < 1000 gm birth weight
  – Mean weight: 734 ± 139gm
  – Mean blood out: 71 ± 65 mL
  – Mean blood in: 114 ± 176 mL
  – Mean # Tx: 4.5 ± 4.6

Blood volume of a 734 gm infant is 59 mL
Approach to Minimize Transfusions

- Delay cord clamping
- Limit number and volume of blood draws
  - Use cord blood when possible
  - Run multiple tests per sample
  - Use iSTAT or point of care testing microsampling
  - Use non-invasive monitoring
- Transfusion Guidelines
- Dedicated aliquoted blood packs
- Optimize nutrition
- Recombinant Epo/ESA therapy
More than 3000 patients studied in randomized controlled trials

<table>
<thead>
<tr>
<th>1st Author</th>
<th>Pub Date</th>
<th>Group Studied</th>
<th>N</th>
<th>Epo Dose</th>
<th>Duration of therapy</th>
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<tbody>
<tr>
<td>Arif B</td>
<td>2005</td>
<td>&lt;33 weeks and &lt;1500 gm</td>
<td>292</td>
<td>400 U/kg/wk</td>
<td>6 weeks</td>
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<tr>
<td>Al-Kharfy T</td>
<td>1996</td>
<td>&lt;1250 gm</td>
<td>55</td>
<td>600 U/kg/wk</td>
<td>6 weeks</td>
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<tr>
<td>Augusti M</td>
<td>2002</td>
<td>&lt;1500 gm</td>
<td>28</td>
<td>250-400 U/kg x 3 x/wk</td>
<td>Until discharge</td>
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<tr>
<td>Bechensteen A</td>
<td>1993</td>
<td>900-1400 gm</td>
<td>29</td>
<td>300 U/kg/wk</td>
<td>4 weeks</td>
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<td>Bierer R</td>
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<td>&lt;1000 gm</td>
<td>16</td>
<td>1200 U/kg/wk</td>
<td>until 35 wks gest</td>
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<tr>
<td>Carnielli V</td>
<td>1992</td>
<td>&lt;32 weeks and &lt;1750</td>
<td>22</td>
<td>40-400 U/kg 3x/wk</td>
<td>7 weeks</td>
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<td>Chen J</td>
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<td>80</td>
<td>300 U/kg/week</td>
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<tr>
<td>Donato H</td>
<td>2000</td>
<td>&lt;1250 gm</td>
<td>114</td>
<td>250 U/kg 5x/wk x 2 wk, 250 U/kg 3x/wk</td>
<td>8 weeks</td>
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<tr>
<td>Emmerson AJB</td>
<td>1993</td>
<td>27-33 weeks</td>
<td>24</td>
<td>50-150 U/kg 2x/wk</td>
<td>7 days until discharge</td>
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<td>Giannakopoulos C</td>
<td>1998</td>
<td>&lt;1300 gm</td>
<td>36</td>
<td>900 U/kg/wk</td>
<td>6-8 wks</td>
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<tr>
<td>Gumi-Pause F</td>
<td>2005</td>
<td>&lt;32 weeks or &lt;1500 gm</td>
<td>45</td>
<td>5000 vs 1250U/kg/wk</td>
<td>from start of anemia to 37 wks gest</td>
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<tr>
<td>Haiden N</td>
<td>2005</td>
<td>&lt;800 gm, &lt;32 weeks</td>
<td>40</td>
<td>300/kg/day vs. 700 U/kg 3x/wk (all got Epo)</td>
<td>9 to 130 days (to 40 wks gest)</td>
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<td>Haiden N</td>
<td>2006</td>
<td>&lt;32 weeks and &lt;1300 gm</td>
<td>64</td>
<td>300/kg/day vs. 700 U/kg 3x/wk (all got Epo)</td>
<td>day 2 of life to 40 wks</td>
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<td>Khatazi S</td>
<td>2008</td>
<td>1000-1750 gm</td>
<td>40</td>
<td>1000 U/kg/wk</td>
<td>day 2-4 x 4 wks</td>
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<td>Kumar P</td>
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<td>&lt;32 weeks and &lt;1250 gm</td>
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<td>6 weeks</td>
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<tr>
<td>Langer J</td>
<td>2008</td>
<td>&lt;32 weeks, &lt;1250 gm</td>
<td>20</td>
<td>250 IV or SC 3 x wk</td>
<td>not stated</td>
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<td>Lauterbach R</td>
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<td>premature</td>
<td>19</td>
<td>200 vs. 800 U/kg/wk</td>
<td>Until discharge</td>
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<tr>
<td>Maier RF</td>
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<td>&lt;1500 gm</td>
<td>241</td>
<td>750 U/kg/week</td>
<td>day 3 to 42</td>
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<tr>
<td>Maier RF</td>
<td>1998</td>
<td>&lt;1000 gm</td>
<td>184</td>
<td>750 vs 1500 U/kg/wk (250 or 500U/kg tiw)</td>
<td>day 3 to discharge</td>
</tr>
<tr>
<td>Maier RF</td>
<td>2002</td>
<td>&lt;1000 gm</td>
<td>43</td>
<td>1200 U/kg/wk</td>
<td>3 weeks</td>
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<tr>
<td>Messer J</td>
<td>1993</td>
<td>&lt;33 weeks</td>
<td>31</td>
<td>300-900 U/kg/wk</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Meyer MP</td>
<td>1994</td>
<td>&lt;32 weeks</td>
<td>80</td>
<td>600 U/kg/wk</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Meyer MP</td>
<td>2003</td>
<td>&lt;33 weeks and &lt;1700 gm</td>
<td>83</td>
<td>1200 U/kg/wk</td>
<td>Until 34 weeks</td>
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<tr>
<td>Obladen M</td>
<td>1991</td>
<td>1380 gm ± 324</td>
<td>20</td>
<td>1400 U/kg/wk</td>
<td>2 weeks</td>
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<tr>
<td>Ohls RK</td>
<td>1991</td>
<td>26-30 weeks</td>
<td>28</td>
<td>1400 U/kg/wk</td>
<td>3 weeks</td>
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<tr>
<td>Ohls RK</td>
<td>1993</td>
<td>Preterm infants with BPD</td>
<td>15</td>
<td>1400 U/kg/wk</td>
<td>2 weeks</td>
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<tr>
<td>Ohls RK</td>
<td>1995</td>
<td>&lt;1250 gm</td>
<td>24</td>
<td>300 U/kg/wk</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Ohls RK</td>
<td>1997</td>
<td>&lt;750 gm</td>
<td>28</td>
<td>1400 U/kg/wk</td>
<td>14 days</td>
</tr>
</tbody>
</table>
Minimum effective dose is 200 U/kg/dose s.c. t.i.w.

Iron supplementation is required

Reticulocytosis seen in all studies

- rEpo > 600U/kg/wk plus iron, less blood transfusions are required
- Early Epo might be beneficial to ELBW with greater phlebotomy losses
- For late treatment, success is dose dependent (Garcia, meta-analysis)
- Donor exposure of 1 for ELBW infants achieved in European Study
Iron

• Required for erythropoiesis
• Epo Rx with inadequate iron supplementation can lead to iron deficiency and ineffective erythropoiesis
  – Neonates receiving Epo with iron show greater reticulocytosis and Hct compared with Epo alone (Carnelli et al)
• Iron dosing in Epo studies ranges from 2–20 mg/kg/day PO to 1–1.5 mg/kg/day or 5–20 mg/kg/week IV
  – 6-12 mg/kg/day oral iron
  – 6 mg/kg/week IV sufficient iron for erythropoiesis in stable infants, but storage iron becomes depleted in the oral group (Maier et al)
Sample Epo Guidelines

• If patient is clinically stable*
  • Follow Hct and Retic every 2 weeks
    – If Hct $\leq 26\%$ and corrected Retic is $\leq 3$ or % Retic is $\leq 6$, begin ESA**
• Check Hct 2 weeks after initiating Epo, then weekly
  – Continue Epo until Hct $\geq 30$

*Instability is defined as an increased risk for poor oxygen delivery. e.g.:
  Prolonged oxygen desaturation episodes, Persistent lactic acidosis,
  Hypotension requiring treatment (pressors, hydrocortisone, boluses of
  isotonic fluid)

** IV ESA should be used if an IV is in place for other reasons. If no IV is in place,
  subcutaneous dosing is most appropriate.
Administration of Epo or Darbe

- **Epo Dose**
  - 200 U/Kg/day IV (daily)
  - 400 U/kg/day SC, on M, W, F

- **Darbepoetin dose**
  - 10 microgram/kg/dose IV or SC once a week

- **Use with iron:**
  - 1 mg/kg/day IV if NPO
  - 6 mg/kg/day (full-volume feedings)
  - Adjust as needed based on ZnPP/H or ferritin
<table>
<thead>
<tr>
<th></th>
<th>24-29 weeks GA</th>
<th>30-40 weeks GA</th>
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</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>51</td>
<td>59</td>
</tr>
<tr>
<td>ZnPP/H: <strong>Mean ± SD</strong></td>
<td>115 ± 33</td>
<td>84 ± 21</td>
</tr>
<tr>
<td></td>
<td>71-207</td>
<td>43-153</td>
</tr>
<tr>
<td>Reference Range</td>
<td>49-181</td>
<td>43-125</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentile</th>
<th>5&lt;sup&gt;th&lt;/sup&gt;</th>
<th>25&lt;sup&gt;th&lt;/sup&gt;</th>
<th>50&lt;sup&gt;th&lt;/sup&gt;</th>
<th>75&lt;sup&gt;th&lt;/sup&gt;</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term, n=308</td>
<td>40</td>
<td>84</td>
<td>134</td>
<td>200</td>
<td>309</td>
</tr>
<tr>
<td>Pre-term, n=149</td>
<td>35</td>
<td>80</td>
<td>115</td>
<td>170</td>
<td>267</td>
</tr>
</tbody>
</table>

Siddappa et al. Neonatology. 2007;92(2):73-82
Extreme Prematurity

- Approximately 31,000 babies per year are born between 24-0/7 and 27-6/7 weeks of gestation in the U.S.
- Most survive (82%)
- Severe impairment rates in survivors are currently ~20%
- Moderate + severe impairment is ~40%
- Rates of neurodevelopmental impairment have not changed in decades
- More children are surviving impaired


% Survival

Gestational Age (weeks)

50%
Mechanisms of Preterm Brain Injury

✧ Damage to existing tissues
  ➢ IVH
  ➢ Infection/inflammation

✧ Interruption of normal development
  ➢ Abnormal neuronal development/synaptogenesis
  ➢ Interruption of oligodendrocyte development
    ✧ Abnormal myelination
  ➢ Abnormal pruning
Brain Growth and Development

Kapellou et al. 2006 PLoS Med

22 Weeks

28 Weeks

Oligodendrocytes

Cortical Surface area (cm²)
Cerebral volume (cm³)

Gestational age at imaging (weeks)
Why Erythropoietin?

**Acute Effects**

- ↓ Inflammation
- ↓ Apoptosis
- ↓ Oxidative injury

**Improved cell survival**

**Long Term Effects**

- **Erythropoiesis**
  - ↓ Free iron
  - ↑ O2 delivery

- **Angiogenesis**
  - ↑ O2 delivery

- **Neurogenesis**
  - Oligodendrogeneration

**Brain Repair**
Epo Molecular Mechanisms

**EpoR Expressing Cells**
- Neurons
- Astrocytes, oligodendrocytes
- Endothelial Cells
- Microglia

**Epo Producing Cells**
- Astrocytes
- Oligodendrocytes
- Neurons

**Epo**, darbepoetin, carbamyl Epo, asialoEpo, Epo mimetics
A Simplistic View of Epo Function

If Epo is present, cells survive. If Epo is absent, cells undergo apoptosis.

Neurons

Oligodendrocytes

Jantzie, 2013; Xu 2012

Epo Receptor Density

No Epo

Epo

Apoptosis

Cell survival
Epo for Neonatal Neuroprotection

- Neuroprotective doses range: 1000 to 5000 U/kg/dose

- Multiple Epo doses provide optimal benefit histologically and functionally

- 40-79% decrease in size of infarction
  - Gonzalez. Dev Neurosci 2009

Vehicle  3 doses Epo
**Epo Risks**

- **Risks (adults with chronic renal failure)**
  - Hypertension
  - Clotting
  - Polycythemia/Anemia
  - Seizures
  - Rash
  - Death

These risks have never been reported in infants using low dose Epo.

**Safety of high dose Epo must be established**

- The risk of ROP and hemangiomas must be assessed in preterm infants
- So far Epo appears to be safe in preterm and term newborns

Phase I/II Trial in ELBW Infants

60 ELBW infants born < 28 weeks and < 1000 grams
Evaluated safety and pharmacokinetics after Epo doses

Safety

- No harmful effects on:
  - ROP
  - Blood pressure
  - Clotting disorders
  - Hemorrhage
  - Kidney function
  - Liver function
  - Sepsis
  - Transfusions
  - Hospital stay

**Epo Pharmacokinetics**

- 2500 U/kg
- 1000 U/kg
- 500 U/kg

*Erythropoietin mU/mL vs. Time (Hours)*

*Pediatrics* 122, 383, 2008
Neonatal Rat vs. ELBW pharmacokinetics

### Neonatal Rodents vs. Preterm Infants < 1000 gm

<table>
<thead>
<tr>
<th>Epo Dose</th>
<th>5000 U/kg s.c.</th>
<th>5000 U/kg i.p.</th>
<th>500 U/kg</th>
<th>1000 U/kg</th>
<th>2500 U/kg</th>
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</thead>
<tbody>
<tr>
<td><strong>AUC (U*h)/L</strong></td>
<td>117,677</td>
<td>140,331</td>
<td>31,412 ± 2780</td>
<td>81,498 ± 7067</td>
<td>317,881 ± 22,941</td>
</tr>
<tr>
<td><strong>Cmax (U/L)</strong></td>
<td>6,224</td>
<td>10,015</td>
<td>8078 ± 538</td>
<td>14017 ± 1293</td>
<td>46467 ± 2987</td>
</tr>
<tr>
<td><strong>T1/2 (h)</strong></td>
<td>8.4</td>
<td>6.7</td>
<td>5.4 ± 0.6</td>
<td>7.1 ± 0.7</td>
<td>8.7 ± 1.4</td>
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</tbody>
</table>
Follow up of these babies

STUDY DESIGN:
- Retrospective analysis
- 17/25 Epo treated babies followed
- 18/26 Controls followed

RESULTS: Epo correlated with improvement of:
- Cognitive (R= 0.22, P = 0.044)
- Motor (R = 0.15, P = 0.026)
- No negative long-term effects of Epo were evident

Suggests that Epo treatment is safe and correlates with modest improvement of neurodevelopmental outcomes

McAdams J Perinatol, June 2012
Swiss Phase III Study

- 440 subjects 26-32 weeks gestation enrolled
  - 3000 U/kg x 3 doses
  - 36 week PMA MRI shows improved WM and DTI
    - Leuchter. JAMA. 2014;312:817-824
  - No safety issues
  - Follow up neurodevelopmental assessments disappointing
Cognitive Outcomes of Preterm Infants Randomized to 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

## Bayley Scales of Infant Development and Neurodevelopmental Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Darbe N=27</th>
<th>Epo N=29</th>
<th>ESA N=56</th>
<th>Placebo N=24</th>
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</thead>
<tbody>
<tr>
<td>Composite cognitive score</td>
<td>96.2 ± 7.3</td>
<td>97.9 ± 14.3</td>
<td>96.5 ± 11.2</td>
<td>88.7 ± 13.5</td>
</tr>
<tr>
<td>NDI</td>
<td>3 (11.1%)</td>
<td>4 (13.8%)</td>
<td>7 (12.5%)</td>
<td>10 (41.7%)</td>
</tr>
<tr>
<td>NDI or Death</td>
<td>4 (14.3%)</td>
<td>5 (16.7%)</td>
<td>9 (15.5%)</td>
<td>13 (48.2%)</td>
</tr>
<tr>
<td>Cerebral Palsy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (20.8%)</td>
</tr>
</tbody>
</table>
PENUT Trial

Preterm Epo Neuroprotection

- Multi-center randomized, placebo-controlled, phase III trial
- 940 infants will be enrolled to evaluate 752 infants (376/arm) at 24-26 months

www.penut-trial.org
We hypothesize that early neonatal high dose Epo treatment of extremely low gestational age neonates will:

- Decrease the combined outcome of *death or severe neurodevelopmental impairment (NDI)* from 40% to 30%

and

- Decrease the combined outcome of *death, moderate or severe neurodevelopmental impairment (NDI)* from 60% to 40% measured at 24-26 months corrected age
Secondary Hypotheses

Neonatal Epo treatment will:

1) Be safe

2) Decrease serial inflammatory cytokines and biomarkers of brain injury

3) Be associated with improved structural integrity of brain as measured by MRI at 36 weeks postmenstrual age
PENUT Trial
Preterm Epo Neuroprotection

UW and SCH NICUs

PENUT Sites 2016 (18, 29 hospitals)
Inclusion Criteria

• NICU inpatients born between 24-0/7 and 27-6/7 weeks of gestation
• Less than 24 hours of age
• Arterial or venous access present
• Parental consent

Pre and postnatal consent can be obtained
Exclusion Criteria

- Known major life-threatening anomalies (e.g. fetal diagnosis of brain, cardiac, or renal malformations, or chromosomal anomalies)
- Severe hematopoietic crises such as DIC, twin-twin transfusion such that 1 twin is not eligible due to polycythemia or hydrops
- Polycythemia (hematocrit > 65%)
- Hydrops fetalis
- Known congenital infection such as toxoplasmosis, CMV, rubella or syphilis.
- Prior administration of erythropoietin to the baby
1. Consent
2. Baseline studies:
   Head Ultrasound
   Epo level +
   Circulating biomarkers

Study Groups
1) Placebo Control: saline i.v. x 6 doses followed by sham injections
2) Epo: 1000 U/kg/dose x 6 doses i.v. followed by 400 U/kg s.c. 3 times per week until 32-6/7 weeks PMA

Corrected Age in Months

Phone Follow up
Interval social and medical history

Follow up assessment:
Standardized Neurologic exam
Bayley III, M-CHAT
Blood pressure, urine and blood
Progress

PENUT Trial
Preterm Epo Neuroprotection

• 723 / 940 enrolled! (77%)
### SAE: Potentially Epo related

<table>
<thead>
<tr>
<th>Event</th>
<th>N (n/N %)</th>
<th>Expected Rate*</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>4 (1%)</td>
<td>22%</td>
<td>35%</td>
</tr>
<tr>
<td>Polycythemia (hematocrit &gt; 60%)</td>
<td>2 (0.5%)</td>
<td>Rare &lt;1%</td>
<td>2%</td>
</tr>
<tr>
<td>Major venous or arterial <strong>thrombosis</strong> (clot) not associated with a central line</td>
<td>1 (0.3%)</td>
<td>Rare &lt;5%</td>
<td>10%</td>
</tr>
</tbody>
</table>

### SAE: Potentially Prematurity related

<table>
<thead>
<tr>
<th>Event</th>
<th>N (n/N %)</th>
<th>Expected Rate*</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary Hemorrhage (Severe)</td>
<td>18 (4.3%)</td>
<td>7%</td>
<td>15%</td>
</tr>
<tr>
<td>NEC (Stage 2b or 3)</td>
<td>34 (8.2%)</td>
<td>11% (6% surgical)</td>
<td>25%</td>
</tr>
<tr>
<td>Sepsis (severe)</td>
<td>41 (9.9%)</td>
<td>33%</td>
<td>50%</td>
</tr>
<tr>
<td>Retinopathy of Prematurity (Severe)</td>
<td>17 (5.6%)</td>
<td>7%</td>
<td>14%</td>
</tr>
<tr>
<td>Intracranial hemorrhage (Grade 3 or 4)</td>
<td>50 (12.1%)</td>
<td>16%</td>
<td>25%</td>
</tr>
</tbody>
</table>

### Other SAE: Expected or unexpected

<table>
<thead>
<tr>
<th>Event</th>
<th>N (n/N %)</th>
<th>Expected Rate*</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 week</td>
<td>49 (11.8%)</td>
<td>Approx. 18%</td>
<td>30%</td>
</tr>
<tr>
<td>25 week</td>
<td>23 (24.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 week</td>
<td>11 (10.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 week</td>
<td>7 (6.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 week</td>
<td>8 (7.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 (1.7%)</td>
<td>&lt; 10%</td>
<td>15%</td>
</tr>
<tr>
<td>Other <strong>unexpected</strong> life threatening event</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 (6.5%)</td>
<td>&lt; 10%</td>
<td>15%</td>
</tr>
</tbody>
</table>
It takes a village...

- Division of Neonatology Faculty and Fellows

- PENUT CCC Team
  - Dennis Mayock
  - Chris Traudt
  - Stephanie Hauge
  - Elizabeth Howland
  - Amy Silva
  - Samantha Walker

- PENUT DCC Team
  - Patrick Heagerty
  - Bryan Comstock
  - Chris Nefcy

- All Site PI’s and Staff
- Nursing Staff
- Pharmacy
- Lab medicine
- Neuroradiology
- Nutrition

The babies and families who are our inspiration
Hypoxic Ischemic Encephalopathy (HIE)

- HIE—1-3/1000 live births
  - 7,000 to 12,000 infants each year in the U.S.
  - 22% of neonatal deaths worldwide
  - Untreated, death or moderate to severe NDI: 65%
Mechanisms of Injury

- Cerebral blood flow
  - Energy failure
  - Glutamate release
  - Calcium-dependent excitotoxicity

- Necrotic cell death
- Delayed cell death

- Apoptotic cell death

Approaches to Therapy

- Receptor antagonists?
- NMDA-2nd messenger modulation

- Inflammation
- Anti-inflammatorics

- Repair
- Restoration

- Plasticity & Recovery
- Stem cells

Antioxidants
- Caspase inhibitors

Growth Factors
- Epo
- Caspase inhibitors
- Antioxidants

Receptor antagonists?
HIE: What are the costs?

- HIE cost per year: $1.7 billion total lifetime costs due to new cases of CP
  - Based on an estimate of 10,000 cases of HIE a year, a 20% rate of CP among the 72% surviving infants with HIE who are treated with HT, with lifetime costs of CP estimated at $1.15 million per individual (2012 currency)

- HIE cost per year: $1.6 billion total lifetime costs due to new cases of intellectual disability
  - Based on CDC cost data and rates of disability derived from HT studies
~50% Cooled Babies *Still* Have Poor Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Cooled</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died or severe disability</td>
<td>44-55%</td>
<td>62-66%</td>
</tr>
<tr>
<td>Died</td>
<td>24-33%</td>
<td>27-38%</td>
</tr>
<tr>
<td>Bayley MDI &lt; 70</td>
<td>25-30%</td>
<td>35-39%</td>
</tr>
<tr>
<td>Bayley PDI &lt; 70</td>
<td>24-30%</td>
<td>34-41%</td>
</tr>
</tbody>
</table>

CLINICAL TRIALS - HIE
1st Clinical Trial of Epo for HIE 2009

Erythropoietin Treatment Improved Neurological Outcome of Newborns with Hypoxic-Ischemic Encephalopathy

Changlian Zhu, MD, PhD1,2, Wenqing Kang, MD3, Falin Xu, MD, PhD1,2, Xiuyong Cheng, MD1,2, Zhan Zhang, MD, PhD2,4, Liting Jia, MD2,4, Ling Ji, MD1, Xiaoyan Guo, MD1, Hong Xiong, MD3, George Simbruner, MD5, Klas Blomgren, MD, PhD6,7, Xiaoyang Wang, MD, PhD1,2

- Epo (n=83) vs. conventional (n=84) treatment
- Epo 300 U/kg (n=52) or 500 U/kg (n=31), Q48h x7
- Epo improved Thompson Score at 7, 14, and 28 days
- Epo decreased the number of MDI scores below 70
- Epo reduced incidence of cerebral palsy at 18 months
- Death or disability at 18 months was:
  - 44% control vs. 25% Epo-treated (p < 0.02)
- No adverse effects of Epo
Prospective case-control study with 45 neonates in 3 groups:

- Normal healthy group: N = 15
- HIE groups: N=15 rEpo 2500 U/kg, s.c. daily x 5 d
  
  N=15 untreated

Results:

- NO ↓ in the HIE-Epo group vs. HIE-control group (P < 0.001)
- EEG improved more in Epo group vs. controls
  - 10 in HIE-Epo group vs 3 in the HIE-control group had normal backgrounds (P=0.01)
- MRI at 3 weeks did not differ between groups
- At 6 months, infants in the HIE-Epo group had fewer neurologic (P = 0.03) and developmental (P= 0.03) abnormalities
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 had 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 exhibited the following outcomes: normal (3), mild language delay (2), mild hemiplegic CP (1) and epilepsy (1)
NEAT O Study

Neonatal Erythropoietin And Therapeutic Hypothermia Outcomes Study

A phase II randomized, placebo-controlled, double masked, multicenter clinical trial of Epo for the treatment of neonatal HIE
NEAT O Study Aims

• Aim 1: Demonstrate feasibility of enrolling and randomizing 50 patients with moderate to severe HIE in a multi-center study, meeting specified recruitment and follow-up goals.

• Aim 2: Demonstrate safety of high-dose Epo in neonates with HIE.

• Aim 3: Correlate brain MRI injury severity with 12 month outcome (AIMS).
NEAT O Study Procedures (N=50)

Study Drug IV: Epo 1000/kg or Saline
Discharge exam

Enrollment < 24 hours
Birth Day 0

Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7

Hypothermia
Baseline MRI

Blood sampling

Postnatal

1wk
6 mo
12 mo

Follow up

Phone

AIMS
WIDEA
Expected vs. Actual Enrollment

Number of Subjects

Expected vs. Actual Enrollment Graph

- Expected
- Actual

January 2014 to January 2015
## Time to Consent

<table>
<thead>
<tr>
<th>Event</th>
<th>N</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Consent</td>
<td>48</td>
<td>12.6 (7.2)</td>
</tr>
<tr>
<td>Consent to 1\textsuperscript{st} dose (hr)</td>
<td>48</td>
<td>3.6 (4.7)</td>
</tr>
<tr>
<td>Age at 1\textsuperscript{st} dose (hr)</td>
<td>50</td>
<td>16.5 (5.9)</td>
</tr>
</tbody>
</table>
Clinical Features

• Severity of encephalopathy
  – Severe: 9 (18%)
  – Moderate: 41 (82%)

• Mean number of study drug doses given = 4.7

• 7 deaths (14%)
  – 44% in severe encephalopathy group
  – 7% moderate encephalopathy group (P=0.02)
    • All death resulted from withdrawal of support
      – Multi-organ failure (6)
      – Severe brain injury, dismal neurologic prognosis (3)
      – Both of above (6)
## Results by Group

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Epo (N=24)</th>
<th>Placebo (N=26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>38.7 ± 1.9</td>
<td>38.7 ± 1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>3556 (618)</td>
<td>3243 (512)</td>
<td>0.06</td>
</tr>
<tr>
<td>10 minute Apgar (mean ± SD)</td>
<td>4.6 ± 1.9</td>
<td>4.9 ± 2.2</td>
<td>0.47</td>
</tr>
<tr>
<td>0-3</td>
<td>19%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>62%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>7-10</td>
<td>14%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Maternal Chorioamnionitis</td>
<td>17%</td>
<td>12%</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Severe encephalopathy</strong></td>
<td><strong>21%</strong></td>
<td><strong>15%</strong></td>
<td><strong>0.72</strong></td>
</tr>
<tr>
<td>Clinical Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In hospital death, n (%)</td>
<td>2 (8%)</td>
<td>5 (19%)</td>
<td>0.42</td>
</tr>
</tbody>
</table>
MRI Scoring system

• Qualitative assessment
• Subcortical injury: injury to the basal ganglia, thalamus or posterior limb of the internal capsule
• White matter
• Cortex
• Cerebellum
• Brain stem
• Scores range from 0 (no signal abnormality) to 3 (widespread injury)
• Each component was scored and summed up to form a total sub-cortical score ranging from 0-72
<table>
<thead>
<tr>
<th>Outcome MRI</th>
<th>Epo (N=23)</th>
<th>Placebo (N=25)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at MRI (days), mean (SD)</strong></td>
<td>5.6 (2.8)</td>
<td>4.9 (1.4)</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Global brain injury score, n (%)</strong></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>None (0)</td>
<td>8 (35%)</td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>Mild (1-11)</td>
<td>14 (61%)</td>
<td>11 (44%)</td>
<td></td>
</tr>
<tr>
<td>Moderate (12-31)</td>
<td>0 (0%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>Severe (≥32)</td>
<td>1 (4.3%)</td>
<td>5 (20%)</td>
<td></td>
</tr>
<tr>
<td><strong>Median [Min, Max]</strong></td>
<td>2 [0-47]</td>
<td>11 [0-70]</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>5.26 (9.9)</td>
<td>16.36 (18.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Presence of brain injury, by region&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcortical&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7 (30%)</td>
<td>17 (68%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cortical</td>
<td>4 (17%)</td>
<td>9 (36%)</td>
<td>0.26</td>
</tr>
<tr>
<td>White matter</td>
<td>12 (52%)</td>
<td>15 (60%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1 (4%)</td>
<td>4 (16%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Cerebellar</td>
<td>0 (0%)</td>
<td>5 (20%)</td>
<td>0.051</td>
</tr>
<tr>
<td>2 or more regions injured</td>
<td>7 (30%)</td>
<td>14 (56%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Outcome: WIDEA</td>
<td>Epo</td>
<td>Placebo</td>
<td>Adjusted treatment effect [95% C.I.](^a)</td>
</tr>
<tr>
<td>----------------</td>
<td>-----</td>
<td>---------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Warner Evaluation (WIDEA)</strong></td>
<td>N=21</td>
<td>N=21</td>
<td></td>
</tr>
<tr>
<td>Age at testing (months)</td>
<td>6.3 (0.6)(^b)</td>
<td>6.1 (0.4)</td>
<td>-</td>
</tr>
<tr>
<td>Total score</td>
<td>75.3 (9.1)</td>
<td>68.8 (10.7)</td>
<td>6.7 [0.69, 12.8]</td>
</tr>
<tr>
<td>Self-care</td>
<td>28.1 (4.2)</td>
<td>26.1 (4.7)</td>
<td>1.8 [-0.97, 4.6]</td>
</tr>
<tr>
<td>Mobility</td>
<td>14.1 (2.7)</td>
<td>12.4 (2.7)</td>
<td>1.5 [0.07, 3.00]</td>
</tr>
<tr>
<td>Communication</td>
<td>16.4 (3.2)</td>
<td>15.3 (2.8)</td>
<td>1.2 [-0.63, 3.1]</td>
</tr>
<tr>
<td>Social</td>
<td>16.7 (4.5)</td>
<td>14.9 (3.2)</td>
<td>2.1 [-0.12, 4.3]</td>
</tr>
</tbody>
</table>

Patients were assessed by phone at 6 months of age.
- Warner Initial Developmental Evaluation (WIDEA)
  - Assesses 4 domains of infant development: self-care, mobility, communication and social cognition
### Warner Evaluation (WIDEA)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Epo</th>
<th>Placebo</th>
<th>Adjusted treatment effect [95% C.I.]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at testing (months)</td>
<td>12.7 (0.9)</td>
<td>12.5 (0.9)</td>
<td>-</td>
<td>0.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total score</td>
<td>122 (14)</td>
<td>110 (31)</td>
<td>10.8 [-2.8, 24.5]</td>
<td>0.15</td>
</tr>
<tr>
<td>Self-care</td>
<td>36.7 (5.1)</td>
<td>33.8 (7.7)</td>
<td>2.8 [-1.1, 6.8]</td>
<td>0.18</td>
</tr>
<tr>
<td>Mobility</td>
<td>28.6 (3.8)</td>
<td>23.8 (8.9)</td>
<td>4.4 [0.46, 8.37]</td>
<td>0.048</td>
</tr>
<tr>
<td>Communication</td>
<td>28.2 (5.1)</td>
<td>25.5 (8.8)</td>
<td>6.0 [-1.9, 6.3]</td>
<td>0.33</td>
</tr>
<tr>
<td>Social</td>
<td>28.8 (6.4)</td>
<td>26.9 (8.9)</td>
<td>1.4 [-0.04, 0.058]</td>
<td>0.57</td>
</tr>
</tbody>
</table>

### Alberta Infant Motor Scale (AIMS)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Epo</th>
<th>Placebo</th>
<th>Adjusted treatment effect [95% C.I.]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head circumference (cm)</td>
<td>45.7 (1.6)</td>
<td>45.5 (2.1)</td>
<td>0.2 [-1.0, 1.4]</td>
<td>0.75</td>
</tr>
</tbody>
</table>

### Growth parameters

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Epo</th>
<th>Placebo</th>
<th>Adjusted treatment effect [95% C.I.]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>9.9 (1.4)</td>
<td>9.7 (1.2)</td>
<td>0.06 [-0.74, 0.86]</td>
<td>0.88</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>74.3 (3.2)</td>
<td>71.7 (6.2)</td>
<td>2.2 [-0.81, 5.3]</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Patients were assessed in person at 12-14 months:
- Warner Initial Developmental Evaluation (WIDEA)
- Alberta Infant Motor Scale (AIMS), a standardized and validated evaluation of infant motor function
<table>
<thead>
<tr>
<th>Serious adverse events</th>
<th>n (%)</th>
<th>n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death during birth hospitalization</td>
<td>2 (8%)</td>
<td>5 (19%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Cardiopulmonary collapse within 2 h of drug</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Thrombosis of major vessel</td>
<td>1 (4%)</td>
<td>1 (7%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Unexpected event related to study drug</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Any of the above</td>
<td>3 (13%)</td>
<td>6 (23%)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Total serious adverse events, n (n per pt.)</strong></td>
<td>4 (0.17)</td>
<td>6 (0.23)</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Other Epo studies for HIE in term infants:

• NeurEpo: J Patkai, France (phase III)
  – N = 330, Epo 1000U/kg + HT
  – Not stratified by severity
  – Stopped due to increased death in Epo group (more severe HIE)

• PAEAN study: H Liley, Australia: (phase III)
  – N= 300, Epo 1000 U/kg; protocol matches HEAL
  – Held while waiting for more info on Patkai study

• A Pappas/S Shankaran: NICHD NRN
  – Phase II/III Epo + HT (proposed study– not yet funded)
WHAT’S NEXT?

HEAL
HIGH-DOSE EPO FOR ASPHYXIA AND ENCEPHALOPATHY
Hypothesis

• High dose Epo given to cooled infants with moderate/severe HIE will reduce the primary outcome of death or NDI at age 22-26 months from 49% to 33%.

• We further hypothesize that neonatal Epo will be safe, will decrease brain injury severity on neonatal MRI, and will decrease serial inflammatory cytokines and biomarkers of brain injury.
Specific Aims

- 500 infants ≥ 36 weeks of gestation with moderate or severe HIE will be enrolled across a network of 17 study sites, in order to evaluate the outcomes of 450 (225 in each arm) infants at 2 years of age.
Birth

Enrollment

1. Consent
2. Randomization
3. Baseline blood draw

Postnatal Days

Epo 1000 U/kg or placebo i.v.

Blood draws

MRI

Study Groups
1) Placebo Control: saline i.v. x 5 doses
2) Epo: 1000 U/kg/dose x 5 doses i.v.

Corrected Age in Months

Phone Follow up
Interval social and medical history, WIDEA-FS

Follow up assessment:
Standardized Neurologic exam
Bayley III, M-CHAT

Discharge

Is it Neuroprotective?
By what mechanisms? Is it reproducible?

Early 2000’s
Optimal dose? Length of treatment?
Dosing interval? Dosing route?
Animals vs. target population

2008-2012: Phase I/II Is it safe?

2013: Efficacy
Phase III