Persistent Pulmonary Hypertension in the US

- 1.9/1000 infants (range 0.5-6/1000)
- ~10% of all neonates with respiratory failure
- No genetic factors identified
- High morbidity, mortality
- At risk for long-term sequelae
### Inhaled NO and PPHN

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>OI</th>
<th>% ECMO</th>
<th>% Mortality</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>NINOS</td>
<td>235</td>
<td>44</td>
<td>55</td>
<td>39*</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Roberts</td>
<td>58</td>
<td>44.4</td>
<td>71</td>
<td>40*</td>
<td>7</td>
<td>7</td>
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<tr>
<td>Clark</td>
<td>248</td>
<td>39</td>
<td>65</td>
<td>48*</td>
<td>8</td>
<td>7</td>
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<tr>
<td>Davidson</td>
<td>155</td>
<td>24.7</td>
<td>34</td>
<td>22</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Konduri</td>
<td>299</td>
<td>19.2</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

### Antenatal Remodeling Associated with PPHN

Normal

PPHN

Pulmonary Artery Relaxations in PPHN

Phosphodiesterases

- Modulate the amplitude and duration of cyclic nucleotide signaling
- Initially described and characterized in 1962, shortly after discovery of cAMP
- Superfamily of 11 primary isoenzymes
**Pulmonary Hypertension and Phosphodiesterase Activity**

PDE

Oxygen
Nitric Oxide

---

**Lung PDE Isoforms**

<table>
<thead>
<tr>
<th>PDE Isoenzyme</th>
<th>GAF Domain</th>
<th>Substrate</th>
<th>Km cAMP</th>
<th>Km cGMP</th>
<th>Specific Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Ca/calmodulin stimulated</td>
<td>1.30</td>
<td>3</td>
<td>Vincocetine</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>cGMP-stimulated</td>
<td>50</td>
<td>50</td>
<td>EHNA</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>cGMP-inhibited, cAMP selective</td>
<td>0.2</td>
<td>0.3</td>
<td>Minimono, enoxalbene, cibostapride</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>cAMP-specific</td>
<td>4</td>
<td>--</td>
<td>Rolipram</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>cGMP-specific</td>
<td>150</td>
<td>1</td>
<td>Sildenafil, tadalafli, vardenafli</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>cGMP-specific</td>
<td>--</td>
<td>0.17</td>
<td>BAY 73-6991</td>
</tr>
</tbody>
</table>

Milrinone: PRIMACORP Trial

**Ductal Ligation Lamb Model**

126-128 day gestation pregnant ewe

- Fetal exteriorization
- Lateral thoracotomy

Ligation of Ductus arteriosus

Cesarean delivery 8-13 days after ligation

**24-hour Ventilation Studies**

- **Servo 300 Ventilator**: Tidal Volume ~ 10mL/kg
- **ABG, Electrolytes and Hct Analysis**
- **IV Infusion of D10W with**
  - 25 mEq/L NaCl
  - 20mEq/L KCl
  - 10mEq/L NaHCO3
- **BP, HR and SpO2**
- **100% Oxygen x 24 hours**
- **Arterial Blood Gases and arterial to Alveolar PO2 ratios (a/A ratios)**
**PPHN Lambs: Milrinone Enhances Relaxations to Prostacyclin**

![Graph showing the effect of Milrinone on prostacyclin relaxations in PPHN lambs.](image1)

*Note: Graph comparison between Control, PPHN, Control + Milrinone, and PPHN + Milrinone.*

**PDE3 Activity After Birth**

![Graph illustrating PDE3 activity changes after birth.](image2)

*Note: Graph comparing PDE3 activity levels between Fetus, 1dSB, 21% O2, 100% O2, and 100% O2-NOS.*

*Sources: Lakshminrusimha et al; Pediatr Crit Care Med, 2009; Chen et al; Pediatr Res 2009*
PDE3 Activity After Birth

Chen et al; Pediatr Res 2009

Mechanically Ventilated

Chen et al; Pediatr Res 2009
PDE3 Inhibition in Lamb PA

Chen et al; Pediatr Res 2009
Milrinone May Enhance iNO Effects

McNamara et al; J Crit Care, 2006
**PDE5 Inhibition: Rationale**

- Enhancement of NO effect
- Protection from NO Toxicity
- Protection from rebound pulmonary hypertension
- Potential for pulmonary vasodilatation

**PPHN Therapies**

- Optimal lung recruitment
  - High frequency ventilation
  - Surfactant
- Pulmonary Vasodilation:
  - Oxygen
  - Inhaled Nitric Oxide
- Cardiovascular support
- ECMO
100% Oxygen Blunts Response to NO

Lakshminrusimha et al; Pediatr Res 2007

Birth-Related Stimuli: O₂, Ventilation, Shear Stress

Endothelial Cell

NO Synthase

NO

Guanylate Cyclase

GTP → cGMP → PDE5 → 5’GMP

Smooth Muscle Cell

Vasorelaxation

NO
**Exposure to Hyperoxia Increases ROS in Lamb Lungs**

![Graph showing Fold DHE Fluorescence relative to 1DSB for different oxygen levels: Fatal, 1DSB, 50% O2, and 100% O2.](image)

**RoGFP: A sensor of protein thiol redox status**

![Diagram showing RoGFP in both reduced and oxidized forms under hyperoxia and normoxia conditions.](image)
Exposure to 95% O₂ Increases ROS in FPASMC

FPASMC: 95% O₂ Blunts cGMP Response to NO
FPASMC: 95% O₂ Blunts cGMP Response to NO

Hyperoxia Increases PDE5 Expression and Activity in FPASMC
Intact Lambs: Hyperoxia Increases PDE5 Expression

30 Minutes of Hyperoxia Increases PDE5 Activity
Superoxide Production is Increased in PPHN Lambs

Control  Ductal Ligation

Ventilation with 100% O₂ in PPHN Lambs

ROS
1DSB  Fetus  100% O₂
PPHN

PDE5 Expression

PDE5 Activity

Brennan et al; Circ Res 2003
**Oral Sildenafil for Human PPHN**

![Diagram showing the mechanism of action of Sildenafil and PDE5]

- NO → cGMP → PDE5

**Graph showing Oxygenation Index**

- Baseline: 0
- 6: Sildenafil (60), Placebo (50)
- 12: Sildenafil (50), Placebo (40)
- 24: Sildenafil (40), Placebo (30)
- 36: Sildenafil (30), Placebo (20)

* * *

Baquero et al, Pediatrics, 2006
Lowered in This Month’s 
AJRCCM…..

Intravenous Sildenafil for 
PPHN

- Five centers enrolled 36 neonates with PPHN or hypoxemic respiratory failure in eight ‘step up’ treatment groups.
- Demographic data for infants:
  - 39±2 weeks gestation
  - 3.44 ± 0.51 kg
  - 34±17 hours of age at enrollment
- Of the 36 infants, 29 were enrolled while already receiving inhaled NO.
## Intravenous Sildenafil Dosing

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Loading dose</th>
<th>Maintenance Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>Duration (h)</td>
</tr>
<tr>
<td>1 (n=2)</td>
<td>0.008 ± 0.005</td>
<td>0.03-0.08</td>
</tr>
<tr>
<td>2 (n=4)</td>
<td>0.011 ± 0.0005</td>
<td>0.5</td>
</tr>
<tr>
<td>3 (n=4)</td>
<td>0.027 ± 0.0029</td>
<td>0.5</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>0.056 ± 0.006</td>
<td>0.5</td>
</tr>
<tr>
<td>5 (n=5)</td>
<td>0.117 ± 0.014</td>
<td>0.5</td>
</tr>
<tr>
<td>6 (n=6)</td>
<td>0.243 ± 0.03</td>
<td>0.5-1</td>
</tr>
<tr>
<td>7 (n=5)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>8 (n=4)</td>
<td>0.427 ± 0.046</td>
<td>3</td>
</tr>
</tbody>
</table>

## Sildenafil Clearance

![Sildenafil Clearance Graph]

- **Sildenafil Clearance (L/hr)**
- **Post-natal age of neonate (days)**
**Safety Events**

- Most frequently reported adverse event was hypotension (6 infants).
- One infant died 2 hours after beginning sildenafil from bilateral tension pneumothoraces.
- All other infants survived, with one infant requiring ECMO support.
- Four subjects discontinued sildenafil due to serious adverse events; three were hypotension, and one was anomalous pulmonary venous connection.
- Two of these serious adverse events (both hypotension) were judged to be sildenafil-related.
Oxygenation Index Over Time

Response to Sildenafil Infusion without iNO

* p=0.05
† p<0.01
Other Neonatal Indications

- PH associated with bronchopulmonary dysplasia
- Congenital diaphragmatic hernia

Sildenafil and Experimental BPD

Ladha et al. AJRCCM 2005
Sildenafil and Experimental BPD

Mourani et al. J Pediatr 2009

Sildenafil and Congenital Diaphragmatic Hernia

PDE5 Expression During Development

Sanchez et al, Pediatr Res 1998
**Increase in Reactive Nitrogen Species in Ventilated Lambs**

3NT

<table>
<thead>
<tr>
<th>100% O2</th>
<th>NO</th>
</tr>
</thead>
</table>

Lakshminrusimha et al, AJRCCM 2006

**Antioxidants Reverse Hyperoxia-Induced Increases in PDE5 Activity**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Activity</th>
</tr>
</thead>
</table>

Farrow et al, Circ Res 2008
Vascular Effects of rhSOD Treatment of PPHN

Lakshminrusimha et al, AJRCCM 2006
Acute Effects of Inhaled Prostacyclin

Kelly et al; J Pediatr 2002;141: 830

Ventilation with 100% O₂ in PPHN Lambs

ROS

1DSB Fetus 100% O₂ PPHN

eNOS Expression

PDE5 Activity

Kelly et al; J Pediatr 2002;141: 830
Birth-Related Stimuli: O$_2$, Ventilation, Shear Stress

Endothelial Cell

NO Synthase

NO

Guanylate Cyclase

GTP \rightarrow cGMP

PDE5

Vasorelaxation

5’GMP

Smooth Muscle Cell
Effect of Cyclic Nucleotides on Phosphodiesterase Activity


IV Sildenafil in Combination with iNO

Birth-Related Stimuli: \(O_2\), Ventilation, Shear Stress

- NO Synthase
  - NO

- Endothelial Cell
  - NO synthase
  - sGC
  - PDE5

- Smooth Muscle Cell
  - Guanylate Cyclase
    - GTP → cGMP
  - PDE5
  - Vasorelaxation
    - 5'GMP

PPHN: NO synthase ↓ sGC ↑ PDE5

Sildenafil