Maternal *Ureaplasma* rDNA Vaccination Improves Neonatal Outcome of *Ureaplasma*-Associated Lung Disease in Mice

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Disclosures

• I have no relevant financial relationships with the manufacturer(s) of any commercial product(s) and/or provider(s) of commercial services discussed in this CME activity.

• I do not intend to discuss an unapproved/investigative use of a commercial product/device in my presentation.
Bronchopulmonary Dysplasia

Normal Infant Lung

BPD Infant Lung
- Arrest in lung development
- Alveolar simplification

Husain et al., 1998
Jobe, 1999
Ureaplasma & BPD

- Significant association in 3 Meta-analyses
  - Wang et al. (1995) - 17 studies
  - Schelonka et al. (2005) - 23 studies
  - Lowe et al. (2014) - 39 studies

- *Ureaplasma* contributes to lung inflammation, altered development & fibrosis
  - Viscardi (2014)

- Our mouse model: *Ureaplasma* and oxygen exposure increased death, lung injury & inflammation
  - Walls et al. (2009)
Ureaplasma Treatment

- Antibiotics may be effective in preventing BPD
  - Clinical
    - Viscardi et al. (2013)
    - Ballard et al. (2011)
    - Ozdemir et al. (2011)
  - Animal
    - Walls et al. (2009)

- Associations with adverse events
- More studies are needed for optimal dosing & safety

- Recombinant DNA (rDNA) vaccine based on the Multiple Banded Antigen (MBA) gene
  - Conserved segment of MBA gene inserted into pVax1 Vector
  - Kong et al. (2012)
Primary Hypothesis

Maternal *Ureaplasma* rDNA vaccination, initiated prior to pregnancy, will improve survival and decrease lung disease severity in a suckling mouse model.
Exploratory Hypothesis

In our model of *Ureaplasma*-associated lung disease, maternal rDNA vaccination will improve the outcome of male pups, which we speculate may otherwise be more adversely effected.
Methods
Dam Vaccination & Pup Lung Disease Model

- Female 6 wk old FVB Mice
- DNA Vaccinated 6, 8, 10 wks
- Time-Mated
- Saline Vaccinated 6, 8, 10 wks
- DNA Vaccinated e14
- Saline Vaccinated e14
- Pups Ureaplasma Infected & Oxygen Exposed
- Survival
- Growth
Methods
Dam Outcomes

- Female 6wk old FVB Mice
- DNA Vaccinated
- Saline Vaccinated
- Serum Antibody
- IgG
- IgA
- Neutralizing
Methods
Pup Outcomes: 3 & 14 Days

- Surviving Pups
  - Born to DNA Vaccinated
  - Born to Saline Vaccinated

- Ureaplasma Culture/PCR
- Serum Antibody
- Lung Weights
- Lung Pathology
- Gender PCR

- IgG
- IgA
- Neutralizing
- H&E
- Immunohistochemistry
- Morphometry

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DNA Vaccination Increases Dam Anti-*Ureaplasma* IgG & IgA Concentrations
DNA Vaccination Improves Pup Survival
DNA Vaccination Decreases Pup Pulmonary Edema

*\( p = 0.022 \)
DNA Vaccination Decreases Alveolar Inflammation

* All pathology done by a board certified pathologist blinded to groups
DNA Vaccination Decreases Pup Alveolar Simplification

* All pathology done by a board certified pathologist blinded to groups

**Average # Alveoli at 3 & 14 Days**

- **3 Day**
  - Saline: N = 11
  - DNA: N = 12

- **14 Day**
  - Saline: N = 10
  - DNA: N = 12

* p ≤ 0.006

HPF = 40X
Exploratory Analysis: Gender

- Literature suggests worse pulmonary outcomes in males, including increased incidence of BPD

  Clinical
  - Kraybill *et al*. 1989
  - Stevenson *et al*. 2000
  - Thomas *et al*. 2006

  Animal
  - Carey *et al*. 2007
  - Lingappan *et al*. 2013

- This experiment:
  - Trend of more male pups survivors in DNA group (p = 0.15)
## Exploratory Analysis: Gender

<table>
<thead>
<tr>
<th></th>
<th>DNA Pups</th>
<th>Saline Pups</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>N</td>
<td>N = 7</td>
<td>N = 4</td>
</tr>
<tr>
<td>Wet/Dry Lung Weight</td>
<td>4.37</td>
<td>4.50</td>
</tr>
<tr>
<td>Alveolar Inflammation</td>
<td>29%</td>
<td>0%</td>
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<tr>
<td>Alveolar Number</td>
<td>104</td>
<td>90</td>
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<tr>
<td>Area/Alveoli (µm²)</td>
<td>228,775</td>
<td>221,407</td>
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<tr>
<td>Wet/Dry Lung Weight</td>
<td>5.01</td>
<td>4.43</td>
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<tr>
<td>Alveolar Inflammation</td>
<td>100%</td>
<td>50%</td>
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<tr>
<td>Alveolar Number</td>
<td>60</td>
<td>69</td>
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<tr>
<td>Area/Alveoli (µm²)</td>
<td>237,155</td>
<td>275,815</td>
</tr>
</tbody>
</table>
Summary

Significant Effects of *Ureaplasma* rDNA Vaccination

- **Dams:**
  - Increases anti-*Ureaplasma* antibodies

- **Pups:**
  - Improves Survival
  - Decreases Pulmonary Edema
  - Decreases Alveolar Inflammation
  - Decreases Alveolar Simplification
Conclusion

• Maternal *Ureaplasma* rDNA Vaccination Significantly:
  - Improves Survival
  - Decreases *Ureaplasma*-associated Lung Disease

Speculation

• IVIG or a *Ureaplasma*-specific monoclonal antibody with high levels of MBA antibody may prevent or decrease severity of *Ureaplasma*-associated lung disease in high-risk preterm infants

• Male gender may increase alveolar inflammation in *Ureaplasma*-associated lung disease
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**Ureaplasma**

- Prokaryotic Bacteria
  - *Mycoplasma* family, *Mollicutes* class
    - *U. parvum* (*U. urealyticum*):
      - serotypes 1, 3, 6, 14
      - serotypes 2, 4, 5, 7-13
  - Small
    - ~1 μm in diameter
    - 0.75-1 kbp genome
  - Lacks cell wall

Viscardi, 2010
Waites et al., 2012
**Ureaplasma**

- Limited biosynthetic abilities
- **Hydrolyzes urea** to generate metabolic energy
- **Mucosal** association in human host

**Multiple Banded Antigen (MBA):**
- Major surface-exposed lipoprotein
- Major attachment factor
- Major virulence factor
- Predominant antigen recognized by host
- **Conserved portion is present in all species & serotypes**

Kallapur *et al.*, 2013
Sung, 2010
Viscardi, 2010
Pup Lung Inflammation Comparison (H&E)

* All pathology done by a board certified pathologist blinded to groups
H&E Pup Lung Morphometry at 14 Days

Saline Group Pup

DNA Group Pup

*both images taken at 200X

* All pathology done by a board certified pathologist blinded to groups
No Difference in Pup Alveolar Area

* All pathology done by a board certified pathologist blinded to groups
No Effect on Pup Growth

Average Pup Birth Weight and Daily Growth

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<thead>
<tr>
<th></th>
<th>3 Day</th>
<th>14 Day</th>
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<tbody>
<tr>
<td><strong>Average Birth Weight (g)</strong></td>
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<td></td>
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<tr>
<td><strong>DNA</strong></td>
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<tr>
<td><strong>Saline</strong></td>
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<tr>
<th></th>
<th>3 Day</th>
<th>14 Day</th>
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<tr>
<td><strong>Average Daily Growth (g/d)</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>DNA</strong></td>
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<tr>
<td><strong>Saline</strong></td>
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Grams

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Giving Life to Possibility
No Difference in Pup Growth

![Graph showing daily growth rate (mean ± SD) over pup days. The graph compares DNA Vaccinated (N = 49) and Saline Vaccinated (N = 56) groups. The p-value is NS (not significant).]
No Difference in *Ureaplasma* Culture & PCR

- **3 Day Pup *Ureaplasma* +Culture and +PCR**
  - *p* = 0.15

- **3 Day Pup *Ureaplasma* -Culture and +PCR**
  - *p* = 0.5

Legend:
- Blue bars: DNA
- Red bars: Saline
Potential Future Directions

- As preterm delivery in the human neonate results in decreased prenatal transfer of maternal antibody, future studies could entail passive treatment of pups with immunoglobulin

- Compare the impact of maternal *Ureaplasma* rDNA vaccine with antibiotics in *Ureaplasma*-associated lung disease

- Determine the impact of maternal *Ureaplasma* rDNA vaccination on innate, reactive and cellular immunity and its mechanisms; and the activity of local and systemic inflammatory mediators

- Evaluating vaccine and *Ureaplasma* infection in models of BPD with genetic modifications that predispose the animals to infection and/or BPD