Second Annual Neonatal Scientific Workshop at the EMA

Welcome Day 2

September 12th – 13th, 2016
1:00 p.m.  **Session VI: Necrotizing Enterocolitis**  
RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) & MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS

3:00 p.m.  **Concluding Remarks**  
MARK TURNER, INC CO-DIRECTOR

3:15 p.m.  **WORKSHOP ADJOURNED**

4:00 – 8:00 p.m.  **SEATTLE WORKGROUP SESSIONS TO BE HELD AT THE MARRIOTT WITH A WORKING DINNER**  
*Workgroup Session I on BPD*  
*Workgroup Session II on Data*  
*Workgroup Session III on Seizures*
1:00 – 3:00 p.m.  

**Session VI: Necrotizing Enterocolitis**

RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) & MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS

**NEC: State of the Art**

MICHAEL CAPLAN (UNIVERSITY OF CHICAGO)

*Biomarkers and Barriers: Opportunities and Challenges in NEC*

KARL SYLVESTER (STANFORD UNIVERSITY)

**Session VI Panel:**

TAHA KEILANI (SIGMA TAU)

IRJA LUTSAR (PDCO)

PAOLO MANZONI (S. ANNA HOSPITAL, TORINO)

TOKUO MIYAZAWA (SHOWA UNIVERSITY, JAPAN)

JOSEPH NEU (UNIVERSITY OF FLORIDA - GAINESVILLE)

JENNIFER CANVASSER (NEC SOCIETY & PPA)
Neonatal Necrotizing Enterocolitis (NEC)

- NEC is an acute inflammatory and coagulative necrosis of any part of the bowel affecting primarily premature infants in a NICU setting.
- NEC has a worldwide incidence varying between 6-15% (2-22% in individual NICU’s) of babies <1500 grams at birth and is associated with a high mortality and morbidity with often devastating long-term sequelae.
- Pathogenesis is poorly understood but seems to be mostly related to alterations in dysregulation of the inflammatory system and abnormal intestinal bacterial colonization pattern.
- Diagnostic criteria are variable and have poor correlation to prognosis; no commonly accepted biomarkers for diagnosis or treatment outcome have as yet been accepted for clinical use.
- No treatment strategy has been clearly effective as yet.
- Focus has been largely on prevention including preventing premature birth and the use of human milk enteral feedings.
- Regulatory science approaches for treatment and prevention have also been challenging.
Session VI: Necrotizing Enterocolitis

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NEC: State of the Art
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO)

Biomarkers and Barriers: Opportunities and Challenges in NEC
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Applying Regulatory Science to Neonates
Second Annual Scientific Workshop at EMA
Session VI: Necrotizing Enterocolitis

Michael S. Caplan, MD
September 13, 2016
NEC: State of the Art - Objectives

• Define the scope of the problem
• Discuss the pathophysiology of NEC
• Identify approaches to early diagnosis
• Opportunities for prevention and/or treatment
• What are the barriers to efficient product development?
### Table 2. Overall Causes of Death among Extremely Premature Infants, 2000–2011.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total live births</td>
<td>7440</td>
<td>7684</td>
<td>7124</td>
<td>22,248</td>
<td></td>
</tr>
<tr>
<td>Total deaths</td>
<td>2043</td>
<td>2193</td>
<td>1839</td>
<td>6075</td>
<td></td>
</tr>
<tr>
<td>Overall mortality rate per 1000 live births (95% CI)†</td>
<td>275 (264–285)‡</td>
<td>285 (275–295)‡</td>
<td>258 (248–268)‡</td>
<td>273 (267–279)‡</td>
<td>0.003</td>
</tr>
<tr>
<td>Cause-specific mortality rate per 1000 live births (95% CI)†‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td>16 (13–19)</td>
<td>14 (12–17)</td>
<td>13 (10–16)</td>
<td>14 (13–16)</td>
<td>0.31</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>65 (60–71)‡</td>
<td>69 (63–75)‡</td>
<td>56 (51–62)‡</td>
<td>64 (60–67)</td>
<td>0.02</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>18 (15–21)</td>
<td>15 (13–18)</td>
<td>12 (10–15)</td>
<td>15 (13–17)</td>
<td>0.09</td>
</tr>
<tr>
<td>Pulmonary: respiratory distress syndrome plus bronchopulmonary dysplasia</td>
<td>83 (77–90)‡</td>
<td>84 (78–90)‡</td>
<td>68 (63–74)‡</td>
<td>79 (75–82)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Infection</td>
<td>22 (19–25)</td>
<td>24 (21–28)</td>
<td>19 (16–22)</td>
<td>22 (20–24)</td>
<td>0.20</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>23 (20–27)‡</td>
<td>29 (25–33)‡</td>
<td>30 (27–34)‡</td>
<td>28 (25–30)</td>
<td>0.04</td>
</tr>
<tr>
<td>CNS injury¶</td>
<td>7 (5–9)</td>
<td>11 (9–14)</td>
<td>10 (8–13)</td>
<td>9 (8–11)</td>
<td>0.21</td>
</tr>
<tr>
<td>Immaturity</td>
<td>86 (80–93)‡</td>
<td>81 (75–88)</td>
<td>81 (75–88)</td>
<td>83 (79–87)</td>
<td>0.04</td>
</tr>
<tr>
<td>Other</td>
<td>37 (33–42)</td>
<td>40 (36–45)</td>
<td>34 (30–39)</td>
<td>37 (35–40)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Patel et al, NEJM 2015
Mortality etiology depends on post-natal age in premature infants.
NEC Pathophysiology (in the preterm infant)

Altered intestinal microbiome

Unbalanced inflammatory response

↑ TLR4, TLR2, et al
↓ human milk feeds (volume, dose, activity)

NICU environment, catheters, etc

Preterm microenvironment, impaired host defense

Delayed feeding and bacterial colonization, lack of breast exposure

Gut hypoperfusion?

Cellular injury

NEC

↑ IL-8
↓ PAF-AH
↓ IkB/↑ NFkB
Genetic factors

Accentuated pro-inflammatory signaling/prematurity
Can we diagnose NEC early so therapy might influence outcome?

- Frequent radiographs
- Abdominal ultrasound/MRI
- Blood biomarkers
- Stool biomarkers
- Urine biomarkers
- Breath hydrogen or other markers
- Heart rate variability algorithms
Opportunities for NEC prevention and/or treatment

- Human milk
- Exclusive human milk
- Probiotics
- Lactoferrin
- Growth factors
- Human milk oligosaccharides
- Other factors that alter cell injury/permeability/inflammation, etc
  - PUFA, PAF-AH, Inter-alpha inhibitor protein, TLR4 antagonists, etc
Bioactive Factors in Human Milk that Modulate NEC Pathogenesis

- Leukocytes
- Immunoglobulins (IgA, etc)
- Oligosaccharides
- PUFA
- Growth Factors (EGF, HBEGF, TGFβ, EPO, NRG-4)
- Lactoferrin
- Cytokines
- Enzymes (PAF-AH, lysozyme)
- Probiotics
Prospective Trial: Human milk and NEC incidence

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>Formula</th>
<th>Human Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-27</td>
<td>14%</td>
<td>8%</td>
</tr>
<tr>
<td>28-30</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>31-33</td>
<td>4%</td>
<td>0.4%</td>
</tr>
<tr>
<td>34-36</td>
<td>9%</td>
<td>0</td>
</tr>
</tbody>
</table>

Randomized patients: 5% formula vs 1% human milk: Odd’s ratio 4.7, p > 0.05.
Lucas and Cole, Lancet 1990:336;1519
Human milk: dose-dependent decrease in NEC or death

Meinzen-Derr et al, J Perinatol, 2009
Exclusive Human Milk-based diet reduces NEC

Study powered to identify reduction in TPN time; no difference found in primary outcome

Sullivan et al, J Peds, 2010
### Probiotics and NEC: meta-analysis

**Aceti et al, Ital J Peds, 2015**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Probiotic</th>
<th>Control</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
</tr>
<tr>
<td>Al-Hosni, 2012</td>
<td>2</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Bin-Nun, 2005</td>
<td>1</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Braga, 2011</td>
<td>0</td>
<td>119</td>
<td>0</td>
</tr>
<tr>
<td>Costalos, 2003</td>
<td>5</td>
<td>51</td>
<td>6</td>
</tr>
<tr>
<td>Dani, 2002</td>
<td>4</td>
<td>295</td>
<td>8</td>
</tr>
<tr>
<td>Demirel, 2013</td>
<td>6</td>
<td>135</td>
<td>7</td>
</tr>
<tr>
<td>Dilli, 2015</td>
<td>2</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>Fernandez-Carrocera, 2013</td>
<td>6</td>
<td>75</td>
<td>12</td>
</tr>
<tr>
<td>Jacobs, 2013</td>
<td>11</td>
<td>548</td>
<td>24</td>
</tr>
<tr>
<td>Kitajima, 1997</td>
<td>0</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Lin, 2005</td>
<td>2</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td>Lin, 2008</td>
<td>4</td>
<td>217</td>
<td>14</td>
</tr>
<tr>
<td>Manzoni, 2006</td>
<td>1</td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td>Mihatsch, 2010</td>
<td>2</td>
<td>84</td>
<td>4</td>
</tr>
<tr>
<td>Mohan, 2006</td>
<td>2</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>Oncel, 2013</td>
<td>8</td>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td>Patole, 2014</td>
<td>0</td>
<td>74</td>
<td>1</td>
</tr>
<tr>
<td>Rojas, 2012</td>
<td>9</td>
<td>372</td>
<td>15</td>
</tr>
<tr>
<td>Rouge, 2009</td>
<td>2</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Roy, 2014</td>
<td>2</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Saengtawesin, 2014</td>
<td>1</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Samanta, 2009</td>
<td>5</td>
<td>91</td>
<td>15</td>
</tr>
<tr>
<td>Sari, 2011</td>
<td>6</td>
<td>110</td>
<td>10</td>
</tr>
<tr>
<td>Serce, 2013</td>
<td>7</td>
<td>104</td>
<td>7</td>
</tr>
<tr>
<td>Strakki, 2007</td>
<td>0</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>Totsu, 2014</td>
<td>0</td>
<td>153</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total (95% CI)**

**Total events**

**Heterogeneity:** Chi² = 20.26, df = 23 (P = 0.63); P = 0%

**Test for overall effect:** Z = 6.08 (P < 0.000001)
# Changing risk ratio/NNT over time on probiotic protection against NEC: meta-analysis results

<table>
<thead>
<tr>
<th>Risk ratio: probiotic v control</th>
<th>Number needed to treat (NNT)</th>
<th>Author</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.32</td>
<td>21</td>
<td>Alfaleh et al</td>
<td>2008</td>
</tr>
<tr>
<td>0.33</td>
<td>22</td>
<td>Wang et al</td>
<td>2012</td>
</tr>
<tr>
<td>0.41</td>
<td>29</td>
<td>Alfaleh et al</td>
<td>2014</td>
</tr>
<tr>
<td>0.47</td>
<td>32</td>
<td>Aceti et al</td>
<td>2015</td>
</tr>
<tr>
<td>0.58</td>
<td>37</td>
<td>Include PiPS (Costeloe et al, 1310 patients, B. breve, (p=NS))</td>
<td>2016</td>
</tr>
</tbody>
</table>
Answered Question: effect size significant, probiotics reduce NEC rate

- UNANSWERED QUESTIONS:
  - Safety in large study with long-term f/u? limited data, so remains unclear
  - Best strain(s), species combination, dose? Combination preparations > Bifidobacteria > Lactobacilli
  - Are all populations the same as the meta-analyses? In US, perhaps not
  - Effect on infection and mortality? Varying results
  - Do meta-analyses predict large RCT results? 30% of the time, large RCT finds opposite results!
  - Appropriate quality control of available product? Key factor in US from the FDA perspective
Compelling preventive strategies with pre-clinical efficacy

- Growth Factors (intestinal maturation and anti-inflammatory effects)
  - EGF
  - HB-EGF
  - TGF-β
  - Neuregulin-4
- Human milk oligosaccharides (n-disialyllacto-N-tetraose)
- Products that reduce cellular injury or inflammation
  - Inter-alpha inhibitor protein, PAF-AH
- Products that alter mucosal permeability/tight junctions
Effect of HB-EGF on NEC in neonatal rats and mice

Table 1: Effect of HB-EGF on the incidence and severity of NEC in newborn rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence of NEC (%)</th>
<th>Histological injury score (median)</th>
<th>Survival rate (%)</th>
<th>Median survival time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>0 (0/10)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTF</td>
<td>10 (1/10)</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHHTF + LPS</td>
<td>65 (13/20)*</td>
<td>2*</td>
<td>25% (5/20)</td>
<td>59</td>
</tr>
<tr>
<td>HHHTF + LPS + HB-EGF</td>
<td>27.3 (6/22)**</td>
<td>1.1**</td>
<td>63.6% (14/22)**</td>
<td>73**</td>
</tr>
</tbody>
</table>

* P < .01 compared with BF or HTF.  
** P < .05 compared with HHHTF + LPS.

Besner Lab, J Ped Surg 2006, 2010
Human milk disialyllacto-N-tetraose protects against NEC in neonatal rats

Jantsher-Krenn and Bode et al; IBD 2014
PAF and PAF-AH in NEC

Stool [PAF] in preterm infants over time
Amer et al, Biol Neo 2004

Controls (top), rPAF-AH (bottom)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Meconium</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 14</th>
<th>NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>48</td>
<td>39</td>
<td>44</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Mean PAF Conc</td>
<td>1.81</td>
<td>1.79</td>
<td>5.14</td>
<td>12.4</td>
<td></td>
</tr>
</tbody>
</table>

Caplan et al, Peds Res 1997

PAF-AH in human serum

- PAF is important mediator in intestinal necrosis
- PAF-AH is deficient in newborns
- PAF receptors are plentiful in gut epithelium
- PAF-AH ko mice develop NEC
- PAF-AH supplementation prevents NEC in newborn rats
- PAF-AH is present in human milk
- PAF-AH could be developed for NEC prevention

Control   | NEC | Death | PAF-AH |
----------|-----|-------|--------|
19/26     | 21/26|       |
6/26 *    | 7/26 *|
NEC prevention: Barriers to efficient product development

- Clarifying/confirming the diagnosis
- Better understanding of the pathophysiology
- NIH and other extramural support for investigators to pursue innovation
- Challenges with powering clinical trials
- FDA challenges
- Orphan drug status
- Pharmaceutical company interest/balance sheet/market assessment
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Applying Regulatory Science to Neonates
Second Annual Scientific Workshop at EMA
Session VI: Necrotizing Enterocolitis

Karl G. Sylvester, M.D.
September 13, 2016
Topics

- **BIOMARKERS**
- Clinical Challenges with NEC and Biomarkers
- What is the landscape of known biomarkers
- What are the challenges of discovering and validating biomarkers
- **UNIFYING HYPOTHESES.**
- Reflect pathophysiology of NEC
- Biomarkers Diagnosis and Screening
- Biomarkers and Prevention
Clinical Spectrum of NEC

• Bell’s I Suspected
  • Limited mucosal injury

• Bell’s II Confirmed
  • Progressive Injury

• Bell’s III Advanced
  • Irreversible injury

Pitfalls: under-treated, over-treated, misdiagnosed

Alternative: objective molecular indicators based upon patient disease biology for tailored / individualized Rx

High specificity (>90%, poor sensitivity <50%)
The problem

Lack of objective diagnostic and prognostic parameters
INSPIRE Network

Glaser - Gerber, Prospective NEC Consortium:

1. Stanford-LPCH
2. Ohio State Univ., NCH
3. Yale New Haven Children’s Hospital
4. Baylor-Texas Children’s Hospital
5. Univ. of Penn., CHOP
6. Johns Hopkins Children’s Hospital
UCSF Children’s Hospital
UCLA, Mattel Children’s Hospital
Boston Children’s Hosp., Harvard

Directors
Larry Moss, MD
Karl Sylvester, MD

Nurse Coordinator
Corinna Bowers

Site PI
Research Nurse

Biologic Studies
Stanford Univ.

Epidemiologic DB
NCH Informatics
Clinical Parameters do not adequately predict outcome in necrotizing enterocolitis: a multi-institutional study

RL Moss, LA Kalish, C Duggan, P Johnston, ML Brandt, JCY Dunn, RA Ehrenkranz, TJaksic, KNobuhara, BJ Simpson, MC McCarthy, KG Sylvester

CRP does NOT correlate with Bell’s Stage

CRP Performed and Results by Bells Stage

<table>
<thead>
<tr>
<th>Bells Stage</th>
<th>Total N</th>
<th>CRP Done</th>
<th>% with CRP</th>
<th>Mean (Min, Max, 95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>246</td>
<td>41</td>
<td>16.7</td>
<td>4.1 (0, 40, 1.7-6.6)</td>
<td>0.904</td>
</tr>
<tr>
<td>IB</td>
<td>71</td>
<td>15</td>
<td>21.1</td>
<td>4.8 (0.1, 16.7, 1.4-8.3)</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>209</td>
<td>30</td>
<td>14.4</td>
<td>3.0 (0.1, 22.0, 0.9-5.1)</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>14</td>
<td>3</td>
<td>21.4</td>
<td>2.5 (0.6, 6.3, -5.6-10.6)</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>52</td>
<td>7</td>
<td>13.5</td>
<td>2.9 (0.1, 11.3, -0.7-6.6)</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>39</td>
<td>6</td>
<td>15.4</td>
<td>2.0 (0.9, 8.5, -1.3-5.5)</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA test was used for this table.

- CRP was not done frequently, averaging between 14-21% of infants for each Bell stage.
- CRP values do not differ significantly among all stages.
Clinical parameters can stratify the patients, but not adequately predict NEC outcomes.

Clinical parameters:

- Patient demographics
- Laboratory tests
- Radiographic analysis
- Medical history
- Physical exam

Ensemble – Integrated Model: Clinical and Molecular Findings

Clinical parameters

Urine peptide markers:
FGA1826;FGA1823, FGA 2659

Clinical

Ensemble

Patient ID after sorted by NEC outcome score
A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives.

- susceptibility/risk biomarker
- diagnostic biomarker
- monitoring biomarker
- prognostic biomarker
- predictive biomarker
- pharmacodynamic/response biomarker
- safety biomarker
REFERENCE- Citations of Biomarkers for NEC and or Sepsis

Published Biomarkers for NEC

- CRP
- IFABP
- PAF
- IP-10
- LFABP
- TNF-δ
- WCC
- IFABP/Cr
- SAA
- Calprotein
- Pro-apoC2/SAA
- Pro-apoC2/Cr
- S100A12

Sensitivity vs. Specificity

- NEC vs. Control
- NEC + Sepsis vs. Con
- NEC Stage I vs. III

Stud...

0 0.2 0.4 0.6 0.8 1

0 0.2 0.4 0.6 0.8 1

0 0.2 0.4 0.6 0.8 1

0 0.2 0.4 0.6 0.8 1
CRP, IFABP, Calprotectin (S100A8,12)

NEC vs. Control
Single time point
A. Based on I-FABPp

B. Based on I-FABPu

Fig 2. Median I-FABP values (after logarithmic transformation), measured from 0–8 h in plasma (A) and urine (B), of 22 NEC versus 15 no-NEC patients.

The positive likelihood ratio is calculated as
\[ LR^+ = \frac{\text{sensitivity}}{1 - \text{spec}} \]
or
\[ LR^+ = \frac{\Pr(T+/D+)}{\Pr(T+/D-)} \]

Relevant Challenges: Treating and Preventing NEC

• Small subject number studies
  • Different controls
  • Different time collections and biologic samples
• Screening studies, baseline values, and biology
• Defining NEC by what criteria; clinical, radiographic, laboratory, treatment
• Contamination by other similar presentation diseases; SIP
• Low prevalence disease
• Multi-center studies
• Ivory tower & study effects of investigating rare diseases—
  • are there significant differences in risk and exposure(s) for NEC in academic
    and non-academic centers?
  • Generalizable
  • Adoption
Domains of NEC Biology & Biomarkers

CRP, Calprotectin, iFABP, IL8

Inflammation

Permeability

Infection

Injury

Citrulline
iFABP, Claudins
Pneumatosis

CRP, Calprotectin, iFABP microbiome
NEC – Clinical Presentation

Prematurity

Metabolism

Nutrition

Microbes

Prematurity
Prevention Strategies

• Feeding Strategies
  • (early v late, slow v. fast)(MBM v formula, banked) (TPN and lipids)

• Probiotics
  • (composition, off target effects, all v some or high risk)
A minimally invasive method to detect intestinal mucosal injury that precedes the onset of fulminant NEC

That reflects the degree of injury
That reflects response to and guides therapy
Newborn Enteropathy

• Metabolic Panel for assessing risk of acquired newborn disease, i.e. Necrotizing Enterocolitis

• Assay of mucosal health
Intestinal injury in neonate vs. juvenile mice after luminal BA-injection

A. Mouse small intestine

14 days

Control

BA-Injected

14 days

Dead cells

28 days

Serosa

B. CBB-stained Gel

C

BA

C

BA

250 (kDa)

150

100

75

50

37

25

20

15

ZZ
Target proteins are abundantly and specifically localized in enterocytes and can be detected in stool if intestines are injured.
Comparison of time-course assays: fecal proteins for a NEC-patient

A.

Infant stool

Healthy control

DOL of NEC (days)

Target protein

NEC initially diagnosed

NEC treatment

B.

Target protein

DOL (days)

Calprotectin

Comparison of time-course assays: fecal proteins for a NEC-patient
However, we caution filing on biomarkers under current patent law. Based on the facts summarized above, an important component for the utility of the invention is its use as a biomarker for diagnostic purposes. There have been some broad changes in the approach that the USPTO takes in the review of such methods since Supreme Court decisions in 2012 and later. In the last few years it has been our experience that it is extremely difficult to persuade Examiners to allow diagnostic claims that were previously routinely granted, and that the lower courts have confirmed the restrictions on patentability.
In *Mayo v. Prometheus*, the U.S. Supreme Court found that claims reciting methods for detecting a correlation between a metabolite and the likelihood of responding to a drug, without "more," are not patentable. 132 S. Ct. 1289 (2012).

In *Association for Molecular Pathology v. U.S. Patent & Trademark Office and Myriad Genetics* ("*Myriad*"), the Court of Appeals for the Federal Circuit found certain method claims ineligible because they were drawn to mental processes. In *Myriad*, one stricken method claim was directed to screening for cancer-predisposing mutations with no further non-mental steps, while another was directed to a method comprising the single step of comparing a gene sequence to a control to identify a certain mutation.

In practice what this has meant is that a **claim directed to a novel correlation for diagnostic or theranostic purposes, which claim uses known reagents and methods, is likely to be rejected** as being drawn to ineligible subject matter. It has been our experience that **only claims with a novel reagent or analytic process; or a claim including treatment steps, are currently considered to be patent eligible.**
Biomarker Clinical Utility

Diagnostic, Prognostic, Monitor Rx Response

Disease A

Disease B

Disease C  Symptom -

Confusing Symptoms

Different clinical outcomes

Drug response

Drug response

Therapeutic Monitoring

Diagnostic Window

Prognostic Window

Biomarker +

Biomarker +

Biomarker +

Symptom +
Potential Projects for Furthering Research in Necrotizing Enterocolitis in Neonates

1) Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?

2) Identification and utilization of biomarkers for the response to treatment of NEC; possibly prognostic indicators.

3) Detailed review and meta-analysis of current methods to prevent and treat NEC in high risk neonates leading to prioritization and study of leading candidates

4) Epidemiologic study of NEC across the globe

5) Determination and clarification of NEC diagnosis: are there different categories that should be considered?
Thank you
Session VI: Necrotizing Enterocolitis

RON PORTMAN, INC CO-DIRECTOR (NOVARTIS) & MICHAEL CAPLAN (UNIVERSITY OF CHICAGO), CO-CHAIRS

NEC: State of the Art
MICHAEL CAPLAN (UNIVERSITY OF CHICAGO)

Biomarkers and Barriers: Opportunities and Challenges in NEC
KARL SYLVESTER (STANFORD UNIVERSITY)

Session VI Panel:
TAHA KEILANI (SIGMA TAU)
IRJA LUTSAR (PDCO)
PAOLO MANZONI (S. ANNA HOSPITAL, TORINO)
TOKUO MIYAZAWA (SHOWA UNIVERSITY, JAPAN)
JOSEPH NEU (UNIVERSITY OF FLORIDA - GAINESVILLE)
JENNIFER CANVASSER (NEC SOCIETY & PPA)
Sigma-Tau Pharmaceuticals, Inc.
Live Biotherapeutics
STP206

Taha Keilani, MD
V.P., Chief Medical Officer
September 13, 2016
STP (Sigma-Tau Pharmaceuticals) Experience

- Introductions
- Study Drug
- IND
  - Pre-IND activities
  - Manufacturing and product release
  - Clinical assays
  - Clinical development Plan
- Current status and plan
• Contains 2 commonly known and used bacteria in food production (*Lactobacilli* and *Bifidobacteria*)

• These bacteria are normal inhabitants of the human gastrointestinal tract, oral cavity, skin, and the vagina

• Associated with a long history of safe use in humans

• Integral to the production of fermented foods and have been consumed safely as part of these foods for millennia

• Are generally considered to be harmless and thus are afforded the generally recognized as safe (GRAS) status
Pre-IND activities
- Preclinical testing
- Toxicology
- Discovering the road to test STP206 in target population
- The need to test the product in older population first?
- Implication on the Clinical Development Plan

Manufacturing considerations
- Finding the manufacturing vendor
- cGMP conditions
- Releasing the product

Clinical Assay development and validation (for identifying the STP206 strains)
• Proposed indication
  • Prevention of Necrotizing Enterocolitis (NEC) in premature babies with birthweight <1500 grams

• The IND submitted (May 18th, 2009)

• Main issues identified:
  • Additional and extensive release testing for objectionable organisms
  • Clinical assay issues
  • Other protocol issues
  • Develop manufacturing process (cGMP)
  • Optimize manufacturing process to obtain target viable count

• IND cleared for the healthy volunteer study on Feb. 12th, 2010
• This study initiated to include the target population

• First introduction and discussion of STP206-002 study protocol with FDA was in July, 2011

• Protocol was finalized in Dec. 2012

• In March, 2013, more pathogens were added for product release testing
Overall Experience and Current Status

**Challenges:**

- Very long time to agree on the IND (started in 2008)
- Communication and corresponding with FDA
- Manufacturing challenges
- At the time of IND submission, no clear Regulatory guidance was available for Live Biotherapeutics

**Current Status:**

- Currently focusing on completing the STP206-002 study
- STP is eager to propose and discuss an expedited path forward for approval
NEC and Regulatory Science
Irja Lutsar MD, PhD
PDCO
University of Tartu, Estonia
Background and current status

• Which disease category is NEC?
  • Infectious disease and treated with antibiotics
    • guidelines for antibiotics
  • Gastroenteral disease
    • guidelines GI medicines
  • Both?

• No diseases with similar mechanisms in adults or older children

• Medicines/drugs could be used and thus regulated
  • For prevention of NEC
  • For treatment of NEC
Current status

- Pathomechanisms and thus management of NEC largely unknown
- No regulatory guidelines on development medicines for NEC
- No PIPs submitted with the indication of prevention or treatment of NEC
- 16 PIPs agreed/under review for antibiotics for LOS (NEC not mentioned)
- No biomarkers identified
  - For diagnosis
  - For treatment
- NEC not mentioned in the neonatal guidelines
Probiotics and lactoferrin for NEC

• No clear position
  • Is it probiotic or pharmabiotic
  • Lactoferrin – drug or dietary supplement
  • Who should regulate approval - EFSA or EMA
    • Food/dietary supplements are regulated by EFSA
    • Medicines are regulated by EMA

• Current regulatory status
  • Probiotics have been presented for scientific advice
  • 1 or 2 PIPs for probiotics (not for NEC)
  • PIP for fecal transplantation (not for NEC)
  • Several academic trials on NEC completed ongoing but no PIPs or regulatory submission
Future directions

- Define management of NEC
- Initiate discussion on regulatory approach on NEC
- If medicines are needed for NEC the regulatory path should be developed
- Regulation of biomarkers for NEC
  - Diagnostic measurement
  - Outcome measurement
The Current Situation of Necrotizing Enterocolitis in Japan

Tokuo Miyazawa
Department of Pediatrics
Showa University School of Medicine
Mortality Rates of ELBW infants between 2000 and 2010
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Infants Born Alive</th>
<th>Neonatal Mortality Rate</th>
<th>Mortality Rate During the NICU Stay</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>2798</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>3065</td>
<td>17.7</td>
<td>17.0</td>
</tr>
<tr>
<td>2010</td>
<td>3070</td>
<td>12.2</td>
<td>13.0</td>
</tr>
</tbody>
</table>

(This national survey covers over 95% of ELBW infants reported in the maternal and health statistics in Japan in each year)
Mortality Rates of ELBW infants between 2000 and 2010
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)

Comparison according to BW

Comparison according to Gestational Age

(This national survey covers over 95% of ELBWI reported in the maternal and health statistics in Japan in each year)
Ranking of Causes of Death during the NICU stay
(National Survey by Committee of Neonatal Medicine, Japan Pediatric Society)

- **Sepsis**: 21% (2010), 25% (2005)
- **NEC/Intestinal Perforation**: 7% (2010), 13% (2005)
- **Circulatory Failure**: 11% (2010), 18% (2005)
- **Congenital Anomaly**: 10% (2010), 10% (2005)
- **Respiratory Failure**: 9% (2010), 11% (2005)
- **CLD**: 4% (2010), 9% (2005)
- **IVH**: 8% (2010), 11% (2005)
- **Severe Asphixia**: 7% (2010), 10% (2005)
- **Others**: 4% (2010), 5% (2005)
Incidence of NEC (from NRN Japan)

(incidence, %) (cover rate, %)


- Necrotizing Enterocolitis
- Idiopathic Intestinal Perforation
- Total of NEC and IIP

Cover rate of VLBWI
Incidence of NEC and Rate of Death after NEC according to GA (NRN Japan 2003-2012)
## Risk factors affecting NEC

### (multivariable analysis, NRN Japan 2003-2012)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Adjusted OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age (1wk)</td>
<td>0.82</td>
<td>0.75-0.86</td>
</tr>
<tr>
<td>Birth Weight (100g)</td>
<td>0.82</td>
<td>0.76-0.89</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>1.46</td>
<td>1.22-1.75</td>
</tr>
<tr>
<td>Cesarean Section</td>
<td>1.06</td>
<td>0.85-1.31</td>
</tr>
<tr>
<td>Out Born</td>
<td>0.97</td>
<td>0.63-1.49</td>
</tr>
<tr>
<td>Multiple Birth</td>
<td>1.07</td>
<td>0.86-1.32</td>
</tr>
<tr>
<td>SGA</td>
<td>1.05</td>
<td>0.75-1.48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Adjusted OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Hypertension</td>
<td>0.78</td>
<td>0.57-1.05</td>
</tr>
<tr>
<td>P-PROM</td>
<td>0.88</td>
<td>0.72-1.07</td>
</tr>
<tr>
<td>Antenatal Corticosteroids</td>
<td>1.03</td>
<td>0.86-1.23</td>
</tr>
<tr>
<td>Apgar Score 1min</td>
<td>0.94</td>
<td>0.89-0.99</td>
</tr>
<tr>
<td>Apgar Score 5min</td>
<td>1.05</td>
<td>0.98-1.11</td>
</tr>
<tr>
<td>RDS</td>
<td>1.44</td>
<td>1.13-1.83</td>
</tr>
<tr>
<td>PPHN</td>
<td>1.54</td>
<td>1.18-2.03</td>
</tr>
<tr>
<td>Indomethacin for PDA</td>
<td>1.48</td>
<td>1.23-1.78</td>
</tr>
</tbody>
</table>

Subjects: birth weight below 1500g
Exclusion: Congenital anomaly, infants with unknown gestational age or defected data
Morbidity risk of NEC vary with birth weight SD score in SGA-ELBW (NRN Japan)

<table>
<thead>
<tr>
<th>BW SD score</th>
<th>Number of Cases (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; -2.0</td>
<td>1050</td>
</tr>
<tr>
<td>-2.0 to -1.5</td>
<td>443</td>
</tr>
<tr>
<td>-1.5 to -1.0</td>
<td>733</td>
</tr>
<tr>
<td>-1.0 to -0.5</td>
<td>1429</td>
</tr>
<tr>
<td>≥ -0.5</td>
<td>5494</td>
</tr>
</tbody>
</table>

OR adjusted for gestational age, sex, plurality, multiple birth, delivery modes, maternal hypertension, clinical chorioamnionitis, and antenatal steroids

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Control</th>
<th>OR</th>
<th>95% C.I.</th>
<th>Revision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula milk</td>
<td>Donor milk</td>
<td>2.77</td>
<td>1.40-5.46</td>
<td>Quigley, 2014</td>
</tr>
<tr>
<td>Trophic feeding</td>
<td>Enteral fasting</td>
<td>1.07</td>
<td>0.67-1.70</td>
<td>Morgan, 2013</td>
</tr>
<tr>
<td>Delayed advancement (after Day 5~7)</td>
<td>Early advancement</td>
<td>0.93</td>
<td>0.64-1.38</td>
<td>Morgan, 2014</td>
</tr>
<tr>
<td></td>
<td>(within Day 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow advancement (15-20ml/kg/day)</td>
<td>Fast advancement</td>
<td>1.02</td>
<td>0.64-1.62</td>
<td>Morgan, 2015</td>
</tr>
<tr>
<td></td>
<td>(30-40ml/kg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous milk feeding</td>
<td>Intermittent bolus</td>
<td>1.09</td>
<td>0.58-2.07</td>
<td>Premj, 2011</td>
</tr>
<tr>
<td></td>
<td>milk feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Milk Fortification</td>
<td>No Fortification</td>
<td>1.57</td>
<td>0.76-3.23</td>
<td>Bown, 2016</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Placebo</td>
<td>0.43</td>
<td>0.33-0.56</td>
<td>AlFelelh, 2014</td>
</tr>
<tr>
<td>Restricted water intake</td>
<td>Liberal water intake</td>
<td>0.43</td>
<td>0.21-0.87</td>
<td>Bell, 2014</td>
</tr>
</tbody>
</table>
## Nutritional Management and Prevention of NEC (from Cochran Review)

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<th>Revision</th>
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<td>Liberal water intake</td>
<td>0.43</td>
<td>0.21-0.87</td>
<td>Bell, 2014</td>
</tr>
</tbody>
</table>

**Management in JAPAN**

- Formula milk: Donor milk
- Trophic feeding: Enteral fasting
- Delayed advancement (after Day 5~7): Early advancement (within Day 4)
- Slow advancement (15-20ml/kg/day): Fast advancement (30-40ml/kg/day)
- Continuous milk feeding: Intermittent bolus milk feeding
- Human Milk Fortification: No Fortification
- Probiotics: Placebo
- Restricted water intake: Liberal water intake
Feeding Policy for VLBWI

● Trophic Feeding
  • To avoid gut atrophy, colonize normal microbiota, prevent NEC, PNAC and infections.
  • Start with own mother’s milk (if possible), at least within 72 hours after birth.

● Advancement of Enteral Feeding
  • Start at 10ml/kg/d and increase daily by 10-20ml/kg/d, up to 150-160ml/kg/d

● Use of Donor Milk
  • The official human milk banking program is not available in Japan.
    In 2014, the first human milk bank is established at Showa Univ. Koto Toyosu Hospital.
    It does not provide donor milk outside of their NICU yet.
  • 25% of the NICUs traditionally use unpasteurized donor milk after screening for pathogens by checking serum antibodies of the donor mother.
  • If OMM is not available, preterm infant formula is applied in general case.
Other Characteristic (experimental) Management in Japan

- Examination of **C-reactive protein (CRP)** as a biomarker of infectious disease and necrotizing enterocolitis
  
  Pourcyrous M. Pediatrics 2005;116:1064-1069

- Screening of PDA with **daily echocardiography by neonatologists**
  
  Roze JC. JAMA 2015;313:2441-2448

- Routine **administration of enema** to prevent feeding intolerance
  - 1ml/kg/dose, 1 to 3 times per day

- Comparatively **Restricted Water Intake**
  - Start at 60ml/kg/day and increase daily by 10ml/kg/day
  - Increase up to 120 (enteral and parenteral)-150 (enteral feeding only)
High Concentration of DHA Level in Human Milk of Japanese Mothers

Human Milk Fatty Acid Composition from Nine Countries Varies Most in DHA

Rebecca Yuhas*, Kathryn Pramuk, and Eric L. Lien
Department of Nutrition Research, Wyeth Nutrition, 500 Arcola Road, Collegeville, Pennsylvania 19426

Lipids, Vol 41(9), 851-858 (2006)

Subjects:
Healthy, nonsmoking mothers (age 14 to 41yr), exclusively breastfeeding single-birth, full-term infants aged 1 to 12 month. Approximately 50 samples were collected from each countries.
### Omega-3 LC-PUFA supplementation and NEC

#### RCTs including preterm infants ≤ 37 weeks

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental Events</th>
<th>Total Events</th>
<th>Total Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clandinin 2005</td>
<td>13</td>
<td>242</td>
<td>3</td>
<td>2.13 [0.62–7.33]</td>
</tr>
<tr>
<td>Fewtrell 2002</td>
<td>5</td>
<td>95</td>
<td>2</td>
<td>2.63 [0.52–13.24]</td>
</tr>
<tr>
<td>Fewtrell 2004</td>
<td>5</td>
<td>122</td>
<td>2</td>
<td>2.38 [0.47–12.01]</td>
</tr>
<tr>
<td>Harper 2010</td>
<td>3</td>
<td>427</td>
<td>4</td>
<td>0.72 [0.16–3.20]</td>
</tr>
<tr>
<td>Makridas 2009;Manley 2011</td>
<td>14</td>
<td>322</td>
<td>7</td>
<td>2.06 [0.85–5.09]</td>
</tr>
<tr>
<td>Vanderhoof 1999</td>
<td>2</td>
<td>77</td>
<td>2</td>
<td>1.01 [0.15–7.01]</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td><strong>1285</strong></td>
<td><strong>1158</strong></td>
<td><strong>51.5%</strong></td>
<td><strong>1.80 [1.06–3.07]</strong></td>
</tr>
</tbody>
</table>

Heterogeneity: \( \chi^2 = 2.29, \text{df} = 5 (P = .81); I^2 = 0\%

Test for overall effect: \( Z = 2.17 (P = .03) \)

#### RCTs including preterm infants ≤ 32 weeks

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental Events</th>
<th>Total Events</th>
<th>Total Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlson 1998</td>
<td>1</td>
<td>34</td>
<td>15</td>
<td>0.17 [0.02–1.21]</td>
</tr>
<tr>
<td>Groh Wargo 2005</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Henriksen 2008</td>
<td>1</td>
<td>68</td>
<td>2</td>
<td>0.54 [0.05–5.79]</td>
</tr>
<tr>
<td>Innis 2002</td>
<td>1</td>
<td>53</td>
<td>2</td>
<td>1.08 [0.10–11.70]</td>
</tr>
<tr>
<td>O'Connor 2001</td>
<td>9</td>
<td>278</td>
<td>6</td>
<td>0.77 [0.28–2.11]</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td><strong>468</strong></td>
<td><strong>432</strong></td>
<td><strong>48.5%</strong></td>
<td><strong>0.50 [0.23–1.10]</strong></td>
</tr>
</tbody>
</table>

Heterogeneity: \( \chi^2 = 2.26, \text{df} = 3 (P = .52); I^2 = 0\%

Test for overall effect: \( Z = 1.72 (P = .08) \)

**Total (95% CI)**  
1753 | 1590 | 100.0% | 1.17 [0.77–1.79]  
45

Heterogeneity: \( \chi^2 = 9.41, \text{df} = 9 (P = .40); I^2 = 4\%

Test for overall effect: \( Z = 0.75 (P = .46) \)

Test for subgroup differences: \( \chi^2 = 7.02, \text{df} = 1 (P = .008), I^2 = 85.7\% \)

---

**Omega-3 Long-Chain Polynsaturated Fatty Acids for Extremely Preterm Infants: A Systematic Review**

Peiyin Zhang, Pascal M. Lavoie, Thierry Lacaze-Masmonteil, Marc Rhaïs and Isabelle Marc

*Pediatrics* 2014;134;120; originally published online June 9, 2014; DOI: 10.1542/peds.2014-0459
Summary

• NEC still has a considerable impact mortality of ELBWIs, even though low incidence in Japan (1.6%)

• The exact reason underlying the low incidence of NEC are poorly understood.

• Some of the traditional, experimental management practices in Japan may account for low incidence of NEC.

• The difference of human milk composition (and enterobacterial flora), attributed to the unique lifestyle habits of Japanese people may contribute to the low incidence of NEC.

• Owing to the insufficient evidence in the regard, further investigation is warranted.
Thank you for your attention!
NEC Society

Jennifer Canvasser, MSW
Founder & Executive Director
Micah, the day before he developed NEC.

Micah’s NEC led to bowel resection and renal failure.

Nine months later, Micah lost his battle.
How to increase awareness, funding & prioritization of NEC?

Family-Patient Engagement

• In the NICU
• In the efforts to drive change
• In mainstream conversations
Engagement in the NICU
Engagement in efforts to drive change
Making NEC a mainstream conversation
Save the date!

NECROTIZING ENTEROCOLITIS SYMPOSIUM
A Transdisciplinary Approach to Improved NEC Outcomes

The NEC Society, in partnership with UC Davis, is proud to present the first national conference on necrotizing enterocolitis (NEC), made possible by a PCORI Engagement Award. The Symposium will bring together the leading clinicians, researchers, parent advocates and others involved in the study and advancement of knowledge in associated NEC, a disease that has shown little advancement in prevention or treatment options in over five decades. CME credits will be available.

April 6 & 7, 2017
On the UC Davis Campus
Contact: Jennifer Canvasser
jennifer@NECSociety.org
www.NECSociety.org

Registration details coming soon!

ATTENDEES
Physicians
Nurse practitioners
Nurses
Family advocates
Physician Assistants
Pharmacists
Dietitians/Nutritionists
Social workers
Policymakers
Nonprofit organizations and other stakeholders

TOPICS
Defining NEC
Early detection of NEC
Intrauterine and NEC
Human milk and NEC
NEC prevention checklist
NEC registry and biorepository
Parent education and involvement
Increasing funding for NEC research
NEC clinical trials
Probiotics and NEC
Animal models of NEC
Treatment options for NEC
NEC and neurodevelopment
Transfusion associated NEC

UC DAVIS
CHILDREN'S HOSPITAL

NEC SOCIETY

MADE POSSIBLE BY A PCORI ENGAGEMENT AWARD
Session VI: Necrotizing Enterocolitis

Paolo Manzoni
Strategies to Reduce Necrotizing Enterocolitis: Use of Lactoferrin and Probiotics
Disclosure

• I have nothing to disclose related to this presentation
The background: Human Milk prevents NEC

Human fresh Milk prevents NEC: the higher the intake, the higher the protection


- Human fresh milk contains probiotics, regardless of geographic areas and feeding.
- An infant fed with 800 ml/day of maternal milk will ingest $10^5$-$10^7$ bacteria every day.
Probiotics and prevention of NEC

Only RCTs including < 37 wks g.a. and/or < 2500g bw.

Twenty-four eligible RCTs

High variability of enrolment criteria, baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics, and feeding regimens.

<table>
<thead>
<tr>
<th>Prevention</th>
<th>RR</th>
<th>95% CI</th>
<th>Nr. of studies</th>
<th>Nr. of infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention of severe NEC (≥ or = stage II)</td>
<td>0.43</td>
<td>0.33-0.56</td>
<td>20</td>
<td>5529</td>
</tr>
<tr>
<td>Prevention of overall mortality</td>
<td>0.65</td>
<td>0.52-0.81</td>
<td>17</td>
<td>5112</td>
</tr>
<tr>
<td>Prevention of nosocomial sepsis</td>
<td>0.91</td>
<td>0.80-1.03</td>
<td>19</td>
<td>5338</td>
</tr>
</tbody>
</table>
Probiotic preparations containing either lactobacillus alone or in combination with bifidobacterium were found to be effective.

No reports of systemic infection with the probiotic supplemental organism.
Summary of the current evidence about Probiotics for prevention of NEC and Mortality

✓ Probiotics (as a category) can significantly prevent / improve:
  1. NEC
  2. all-cause Mortality prior to discharge
  3. time needed to reach full feeds

✓ “The dramatic effect sizes, tight confidence intervals, extremely low P values, and overall evidence indicate that additional placebo-controlled trials are unnecessary if a suitable probiotic product is available” (Deshpande et al, Pediatrics 2010)

✓ The evidence is so striking that the last 2014 Cochrane Review states:
  1. “This updated review of available evidence strongly supports a change in practice”
  2. “Whenever a probiotic product is available, its administration for prevention of NEC is recommended”
Gaps in knowledge - QI Actions about Probiotics for prevention of NEC (as of today)

✔ Which probiotic strain(s)? Single strains, or Mixtures?
  → in most of the NEC studies, *Lactobacillus spp* and *Bifidobacterium spp* have been used
  → mixtures proved effective in most cases
  → A mixture choice (with Lactobacilli and Bifidobacteria) clearly mimics the probiotic’s content of human milk

✔ What dosages?
  → At least 3 x 10^6 CFU/day

✔ When to start? which duration?
  → start as soon as possible to prevent pathological colonization in the gut
  → It seems reasonable to go ahead till full feeds with human milk are tolerated

✔ What are the interactions with human and formula milk?

✔ Are they fully safe?
  → Generally yes. So far, only scattered, anecdotical cases of probiotic sepsis in preterms have been reported
LF is the major whey protein in mammalian milk.

High [77%] structural homology between:

- Bovine LF → extracted and purified by cow’s milk
- Human LF → recombinant engineering: *thalactoferrin*

In the stomach, pepsin digests and releases a potent peptide antibiotic called lactoferricin from native LF.

Human and Bovine LF share the same:

- LACTOFERRICIN (N-terminal, 11-aminoacidic peptide with antimicrobial activity) (*Lupetti 2004*)
- Orally administered LF remains active even after stomach passage
- High intestinal uptake and gut actions (*Lonnerdal 2011*)
Concentrations of LACTOFERRIN decrease in mature human milk vs. colostrum.

This decrease typically occurs in all mammals.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Concentrations of lactoferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman</td>
<td>2 (mature milk) – 6 (colostrum) mg/ml</td>
</tr>
<tr>
<td>Cow</td>
<td>0,2-0,5 mg/ml</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Dog</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Goat</td>
<td>0,2 mg/ml</td>
</tr>
<tr>
<td>Pig</td>
<td>0,2 mg/ml</td>
</tr>
</tbody>
</table>
Why LACTOFERRIN might also prevent NEC? *the rationale*

- LF prevents Late-Onset Sepsis in VLBWs (*Manzoni et al, JAMA 2009*)
- Lactoferrin and lysozyme in breast milk are synergistic, and kill bacteria.
- The antimicrobial characteristics of LF may facilitate a healthy intestinal microbiome → LF is *bifidogenic*, promoting Bifidobacteria and Lactobacilli microflora in the gut → these probiotics prevent NEC (*Alfaleh et al, Cochrane 2014; Deshpande et al, Lancet 2007*)
- LF has *trophic and pro-proliferative activity* on the nascent enterocytes, regulating *gut permeability* (*Buccigrossi et al, Ped Res 2007*)
- LF enhances *anoikis (apoptosis)* of infected enterocytes in the gut (*Sherman et al, Med Hypoth 2005*)
- The immuno-modulatory activates of LF activate dendritic cells (DC) and DCs then induce a *Th1 helper cell population* that resists neonatal infection.
- Lactoferrin has anti-inflammatory actions that may mitigate the proinflammatory state that is present in the gut before the onset of necrotizing enterocolitis (NEC).
  - LF *attenuates oxidation* by suppressing free radical activity, and decreasing levels of oxidative products (*Raghuveer et al, Ped Res 2002*)
2 RCTs retrieved (all with BLF)
552 VLBW infants analysed. Moderate heterogeneity.
R.R. 0.30
NNT 20
Current available evidence graded as “low-to-moderate quality”
THANK YOU FOR YOUR ATTENTION!!

SEE YOU IN TORINO IN 2016!!

6TH ICCN INTERNATIONAL CONFERENCE on CLINICAL NEONATOLOGY

TURIN
Centro Congressi Unione Industriale Torino

September 22nd - 24th 2016

Pre congress Courses: September 21st - 22nd 2016

www.iccn2016.eu
• Backup slides
## LACTOFERRIN trial for prevention of NEC


- After the end of the JAMA study, 7 of 11 Centres [6 in Italy, 1 in New Zealand] agreed on continuing recruitment for an 18-month additional period, with a target enrolment of 800 patients, to achieve significance for the outcome “NEC”.

- Design, Study Protocol, Enrollment criteria and timing, Randomization 1:1:1, LF and LGG dosages were unchanged

<table>
<thead>
<tr>
<th></th>
<th><strong>LF</strong>&lt;br&gt;N=251</th>
<th><strong>PLACEBO</strong>&lt;br&gt;N=259</th>
<th><strong>R.R.</strong></th>
<th><strong>95% C.I.</strong></th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe NEC (&gt;2nd stage)</td>
<td>2.0%</td>
<td>5.4%</td>
<td>0.37</td>
<td>0.14-1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Overall Mortality</td>
<td>2.0%</td>
<td>6.9%</td>
<td>0.28</td>
<td>0.11-0.76</td>
<td>0.007</td>
</tr>
<tr>
<td>NEC and/or Death</td>
<td>4.0%</td>
<td>10.1%</td>
<td>0.39</td>
<td>0.19-0.80</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Absolute risk reduction = 3.41 percent.** **NNT (Number Needed to Treat) = 30**

<table>
<thead>
<tr>
<th></th>
<th><strong>LF + LGG</strong>&lt;br&gt;N=242</th>
<th><strong>PLACEBO</strong>&lt;br&gt;N=259</th>
<th><strong>R.R.</strong></th>
<th><strong>95% C.I.</strong></th>
<th><strong>p-value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe NEC (&gt;2nd stage)</td>
<td>0%</td>
<td>5.4%</td>
<td>0.00</td>
<td>---</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Overall Mortality</td>
<td>3.8%</td>
<td>6.9%</td>
<td>0.53</td>
<td>0.24-1.16</td>
<td>0.11</td>
</tr>
<tr>
<td>NEC &amp;/or Death</td>
<td>3.8%</td>
<td>10.1%</td>
<td>0.37</td>
<td>0.18-0.77</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Absolute risk reduction = 5.41 percent.** **NNT (Number Needed to Treat) = 19**

---

**LACTOFERRIN trial for prevention of NEC**


- After the end of the JAMA study, 7 of 11 Centres [6 in Italy, 1 in New Zealand] agreed on continuing recruitment for an 18-month additional period, with a target enrolment of 800 patients, to achieve significance for the outcome “NEC”.

- Design, Study Protocol, Enrollment criteria and timing, Randomization 1:1:1, LF and LGG dosages were unchanged
Gaps in the current knowledge

- **Dosages** → likely higher than 100 mg/kg, but how higher? Fixed or pro-kg dosage?
- **Dosing/Schedule** → once a day? Or many times a day (mimicking the human milk?)
- **Duration** → in preterms, how long? And in infants, how long and starting when?
- **Interactions with human milk** → better effects when added to HM or to Formula?
- **Interactions with probiotics** → better effects when added to BB or LB strains?
- Short-term and long-term **safety**?
- Any effect on **other outcomes of prematurity** [e.g. ROP, BPD]?
- **Generalizability** of the bovine LF findings
- Generalizability also to Human Recombinant Lactoferrin (*Thalactoferrin*)
Proposed guideline for the use of probiotics in preterm neonates based on the evidence available

- A combination of Lactobacillus and Bifidobacterium is preferred.
- The dose should be at least $3 \times 10^9$ organisms per day
- Starting when the neonate is ready for enteral feeds
- Continued until 35 weeks’ corrected age or discharge

Session VI: Necrotizing Enterocolitis

Josef Neu
Being led astray: 50 years---not much progress

• Lumpng of several diseases called “NEC” into the same data set. Would we do this for diabetes or cancer?
  • Spontaneous intestinal perforations
  • Ischemic bowel associated with heart disease, polycythemia
  • Food protein intolerance
  • “classic” form seen most commonly in preterms

• Animal models that do not represent the disease that we see in most babies who develop NEC.
Is there a Clear Definition of NEC?

Bells is Broken

• Stage 1- Too non-specific and the term should not be used.

• Stage 2- Radiographic signs can be “fuzzy”.

• Stage 3- Free air on radiograph could signify intestinal necrosis or Spontaneous Intestinal Perforation (SIP)
Neu, J. Acta Paediatrica, 2005 94 (Supple 449): 100-105

Pathophysiologic Overview at the Barrier

- Altered microbiota (low diversity caused by antibiotics)
- Intact intercellular junction
- Mucus
- Intestinal necrosis
- Exaggerated inflammation and tissue injury
- Genetics: Polymorphisms in TLRs
- Immature intestinal barrier: Decreased mucus, Decreased IgA, Low intercellular junction integrity and increased permeability

Neu J, Walker WA. NEJM 2011
Causes of Inappropriate Colonization “DYSBIOSIS”

Type of Diet: Human Milk versus Formula

Lack of Enteral Feeding; TPN, Intestinal pH

Antibiotics and Microbial Environment

Type of Diet: Human Milk versus Formula
Fecal Microbiota: NEC
Mai V, Young C. PLOS One, May 2011

- Proportions of the four major phyla two weeks before and the week of diagnosis.
Abundance of Proteobacteria

Most Commonly used Drugs in the NICU: Majority of VLBW infants are Exposed to Antibiotics

Top 10 Medications Prescribed in the NICU

<table>
<thead>
<tr>
<th>Medication</th>
<th>Prescriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>186,799</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>171,388</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>90,152</td>
</tr>
<tr>
<td>Vitamin (multivitamin)</td>
<td>64,329</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>55,455</td>
</tr>
<tr>
<td>Caffeine citrate</td>
<td>48,814</td>
</tr>
<tr>
<td>Furosemide</td>
<td>47,278</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>44,218</td>
</tr>
<tr>
<td>Beractant (Survanta)</td>
<td>36,410</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>27,541</td>
</tr>
</tbody>
</table>
Odds Ratio of NEC with Increased Days on Antibiotics
Alexander, V.N. J. Pediatrics, Sept. 2011

Average length of Treatment increases odds by 50%
Gastric Acid Inhibition

Ranitidine is Associated With Infections, Necrotizing Enterocolitis, and Fatal Outcome in Newborns

WHAT'S KNOWN ON THIS SUBJECT: Although still off-label for newborns, the use of inhibitors of gastric acid secretion continues to increase. Acid-suppressive drugs could facilitate the onset of infections in adults and children. Evidence for efficacy is weak in newborns, particularly if preterm.

WHAT THIS STUDY ADDS: This is the first prospective study demonstrating an association between the use of ranitidine and infections, necrotizing enterocolitis, and fatal outcome in very low birth weight newborns. Caution is advocated in using ranitidine in newborns.

AUTHORS: Gianluca Terrin, MD, PhD; Annalisa Passariello, MD, PhD; Mario De Curtis, MD, PhD; Francesco Manguso, MD, PhD; Gennaro Salvia, MD; Laura Lega, MD; Francesco Messina, MD; Roberto Paludetto, MD; and Roberto Berni Canani, MD, PhD

1Department of Women’s Health and Territorial Medicine, University La Sapienza, Rome, Italy; 2Department of Pediatrics, University Federico II, Naples, Italy; 3Neonatology Unit, Monaldi Hospital, Naples, Italy; 4Department of Pediatrics, University La Sapienza, Rome, Italy; 5Gastroenterology Unit, Cardarelli Hospital, Naples, Italy; 6Neonatology Unit, Fatebenefratelli Hospital, Naples, Italy; 7Neonatology Unit, Meyer Pediatric Hospital, Florence, Italy; 8Neonatology Unit, V Betania Evangelic Hospital, Naples, Italy; and 9European Laboratory for the Investigation of Food Induced Diseases, Naples, Italy
Effect of Total Parenteral Nutrition (TPN) in Mice

# Morbidities: Early vs. Late Feeding

<table>
<thead>
<tr>
<th>Outcomes (%)</th>
<th>Early (n = 79)</th>
<th>Late (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC</td>
<td>6.3</td>
<td>10.0</td>
</tr>
<tr>
<td>ROP</td>
<td>16.7</td>
<td>52.1**</td>
</tr>
<tr>
<td>CLD</td>
<td>21.5</td>
<td>69.4**</td>
</tr>
<tr>
<td>PVL</td>
<td>0.0</td>
<td>6.0*</td>
</tr>
<tr>
<td>IVH</td>
<td>24.1</td>
<td>24.0</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>8.0</td>
<td>25.0**</td>
</tr>
</tbody>
</table>

* Early vs. Late p<0.05;  
** Early vs. Late p<0.0001

Necrotizing Enterocolitis (NEC); Retinopathy of Prematurity (ROP); Chronic Lung Disease (CLD); Periventricular Leukomalacia (PVL); Intraventricular Hemorrhage (IVH); Comorbidities = The presence of 2 or more neonatal outcomes.

Konnikova, et al. PLOS One 2015
Microbial Dose from Human Milk

- Assume intake of 800 ml/day
- Assume $10^{5-6}$ bacterial cells/ml
- This will provide $10^{7-8}$ bacterial cells (personalized?) daily, close to the dose in most probiotic studies.
Recommendations

• Define and Delineate “NEC”

• Proximal components of pathophysiology (environment and intestinal immaturities) are important. Once the cascade has started, it is difficult to stop.

• Focus on prevention—”primum non nocere”.
  • Feed (fresh human milk), limit antibiotics and other drugs known to alter microbes.

• Proximal components of pathophysiology and early recognition of risk are important.
Considering both impact and feasibility, which of the following projects is your first choice?

1. Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?
2. Identification and utilization of biomarkers for the response to treatment of NEC; or possible prognostic indicators.
3. Detailed review and meta-analysis of current methods to prevent and treat NEC in high-risk neonates leading to prioritization and study of leading candidates.
4. Epidemiologic study of NEC across the globe.
5. Determination and clarification of NEC diagnosis: are there different categories that should be considered?
6. “Walk-in Option A” (offered up by audience)
7. None of the above
Considering both impact and feasibility, which of the following projects is your second choice?

1. Identification and utilization of biomarkers for the early diagnosis of NEC; are there candidates available and what additional investigation is needed?
2. Identification and utilization of biomarkers for the response to treatment of NEC; or possible prognostic indicators.
3. Detailed review and meta-analysis of current methods to prevent and treat NEC in high-risk neonates leading to prioritization and study of leading candidates.
4. Epidemiologic study of NEC across the globe.
5. Determination and clarification of NEC diagnosis: are there different categories that should be considered?
6. “Walk-in Option A” (offered up by audience)
7. None of the above
Concluding Remarks

• Mark Turner, INC Co-director
Evening Workgroup Sessions

• Seizures, BPD, Data
• 4-8 pm
• Marriott West India Quay
  • Tamarind - BPD
  • Barbados – Seizures
  • Trinidad - Data
Thank you!