Development of a Low-Dose Challenge Model for Evaluation of Vaccines for Enterotoxigenic *E. coli* (ETEC) in Volunteers


Affiliations:

- Johns Hopkins Bloomberg School of Public Health, Center for Immunization Research, Baltimore, MD
- PATH, Washington DC
- University of Gothenburg, Gothenburg Sweden
Background: Enteric Disease Challenge Models

- Variety of ETEC challenge models evaluated since 1970s
- Most extensively studied strain: ETEC H10407 (Serotype 078:K80:H11)
  - >250 subjects challenged
  - Induces reliable AR at doses \( > 5 \times 10^8 \)
  - Suitable for vaccine efficacy studies: LT, ST, CFA I
ETEC Challenge Models

• Concern that traditional challenge inoculum artificially high relative to natural exposure
  – May lead to false conclusion that candidate vaccine not protective
  – Other bacterial challenge models typically have lower HD$_{50}$

• Historically, lowering H10407 inoculum dose has yielded inconsistent AR
Study Objectives

• Identify an H10407 inoculum dose $\leq 10^8$ that will cause diarrhea in 50% or more subjects

• Determine if recent challenge with lower doses or modified delivery approach still protects upon re-challenge

• Measure mucosal and systemic immune responses in naïve and immune subjects using comprehensive assay array

• Determine if mucosal and systemic immune responses predict protection
Study Design Variables

1. Fasting conditions
   - Overnight fast
     • Animal data suggest increased colonization
     • Observational data suggest higher virulence

2. Buffer
   - Bicarbonate buffer
   - Ceravacx®
     • Rice-based bicarbonate/citrate buffer
     • Equivalent gastric acid buffering
     • Rapidly absorbed in glucose-mediated transport

3. Challenge dose
Study Design

Cohort 1:
1A (n=5) 1x10^8(cfu) with Bicarbonate
1B (n=5) 1x10^8(cfu) with CeraVacx®
1C (n=5) 1x10^7(cfu) with Bicarbonate
1D (n=5) 1x10^7(cfu) with CeraVacx®

If 1x10^8(cfu) is best
Cohort 2A (n=15)
1x10^8(cfu) [optimum buffer]

If 1x10^7(cfu) is best
Cohort 2B (n=15)
1x10^7(cfu) [optimum buffer]
Cohort 2C (n=15)
1x10^6(cfu) [optimum buffer]

If 1x10^7(cfu) is too virulent
Cohort 3A n=10 naïve
Cohort 3B n=10 repeat
[optimum dose]
[optimum buffer]
Methods

- Regulatory approvals obtained January/February 2009
- Recruited healthy volunteers
  - 18-45 yrs
  - No exposure to ETEC, cholera, or LT ≥ 5 years
- Admitted in 3 separate cohorts
  - Cohort 1 February 2009
  - Cohort 2 March 2009
  - Cohort 3 May 2009
- NPO after midnight
- Challenge ~9 hours later
  - 120 mL buffer
  - 30 mL buffer with challenge inoculum
# Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1 N=20</th>
<th>Cohort 2 N=15</th>
<th>Cohort 3 N=10*</th>
<th>TOTAL N=45</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>14 (70%)</td>
<td>11 (73%)</td>
<td>5 (50%)</td>
<td>30 (67%)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>14 (70%)</td>
<td>11 (73%)</td>
<td>9 (90%)</td>
<td>34 (76%)</td>
</tr>
<tr>
<td>White</td>
<td>4 (20%)</td>
<td>4 (27%)</td>
<td>1 (10%)</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (10%)</td>
<td>0</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, yrs</td>
<td>30.3</td>
<td>33.6</td>
<td>29.1</td>
<td>31.1</td>
</tr>
<tr>
<td>Range</td>
<td>19-45</td>
<td>19-43</td>
<td>21-41</td>
<td>19-45</td>
</tr>
</tbody>
</table>

*Includes naïve subjects only. Total number of subjects enrolled in Cohort 3 = 20
Medical Monitoring

• Daily history and physical exam
• Collection and grading of all stools
  – Grade 1: Firm, formed (normal)
  – Grade 2: Soft, formed (normal)
  – Grade 3: Viscous, opaque liquid assuming shape of container
  – Grade 4: Watery, non-viscous opaque liquid
  – Grade 5: Clear or translucent watery or mucoid liquid
• Medical management of clinical signs and symptoms
• Independent Medical Monitor
# Cohort 1 Results

Cohort 1:
1A (n=5) 1x10^8 (cfu) with Bicarbonate
1B (n=5) 1x10^8 (cfu) with Ceravacx®
1C (n=5) 1x10^7 (cfu) with Bicarbonate
1D (n=5) 1x10^7 (cfu) with Ceravacx®

<table>
<thead>
<tr>
<th>H10407 Challenge Dose</th>
<th>Delivery Vehicle</th>
<th>Diarrhea^1(N)/Challenged (N)</th>
<th>Attack Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x10^8 (Cohort 1A)</td>
<td>Bicarbonate</td>
<td>5/5</td>
<td>100</td>
</tr>
<tr>
<td>2x10^8 (Cohort 1B)</td>
<td>Ceravacx®</td>
<td>4/4*</td>
<td>100</td>
</tr>
<tr>
<td>2x10^7 (Cohort 1C)</td>
<td>Bicarbonate</td>
<td>4/5</td>
<td>80</td>
</tr>
<tr>
<td>2x10^7 (Cohort 1D)</td>
<td>Ceravacx®</td>
<td>5/5</td>
<td>100</td>
</tr>
</tbody>
</table>

^1 Diarrhea defined as:
- 1 or more loose stools (> Grade 3) of >300 grams
- 2 or more loose stools (> Grade 3) of >200 grams in a 48 hour period

*One subject withdrawn due to noncompliance
Cohort 2

• Rationale
  – AR similar across groups in Cohort 1

• Strategy
  – Lower dose: $10^7$cfu
  – Traditional buffer: Bicarbonate
Cohort 2 Results

• Confirmed trends observed in Cohort 1
  — Attack Rate ≥50% of challenged subjects
  — Disease severity comparable to higher dose challenge

<table>
<thead>
<tr>
<th>H10407 Dose</th>
<th>Buffer</th>
<th>Severity of Diarrhea¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x10⁷</td>
<td>Bicarbonate</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

¹ Diarrhea defined as:
- 1 or more loose stools (≥ Grade 3) of ≥300 grams
- 2 or more loose stools (≥ Grade 3) of ≥200 grams in a 48 hour period

²Classification based on peak stool number or weight in a 24 hour period
- Moderate: 4-5 stools/24 hrs or 401-800 grams/24 hrs
- Severe: ≥ 6 stools/24 hrs or >800 grams/24 hrs
Cohort 3 Results

Cohort 1:
1A (n=5) $2 \times 10^8$ (cfu) with Bicarbonate
1B (n=5) $2 \times 10^8$ (cfu) with CeraVacx®
1C (n=5) $2 \times 10^7$ (cfu) with Bicarbonate
1D (n=5) $2 \times 10^7$ (cfu) with CeraVacx®

Cohort 2B (n=15)
$2 \times 10^7$ (cfu) with Bicarbonate

Cohort 3A (n=10) Naïve
Cohort 3B (n=10) Re-challenge
$2 \times 10^7$ (cfu) with Bicarbonate
Cohort 3 Results

**Naïve**
- No Diarrhea: N=1
- Mild Diarrhea: N=2
- Mod-Severe Diarrhea: N=7

**Re-Challenge**
- No Diarrhea: N=9
- Mild Diarrhea: N=1
**Combined Outcomes for Subjects Challenged using H10407 Inoculum**

<table>
<thead>
<tr>
<th>Dose (Cohort)</th>
<th>Buffer</th>
<th>Diarrhea</th>
<th>Average Incubation</th>
<th>Early Rx</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2 \times 10^7) (Cohort 1)</td>
<td>Bicarbonate N=5</td>
<td>1 0 4 (80%)</td>
<td>43 hrs</td>
<td>3 (60%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>(2 \times 10^7) (Cohort 1)</td>
<td>CeraVacx® N=5</td>
<td>0 0 5 (100%)</td>
<td>64 hrs</td>
<td>3 (60%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>(2 \times 10^7) (Cohort 2)</td>
<td>Bicarbonate N=15</td>
<td>4 0 11 (73%)</td>
<td>52 hrs</td>
<td>10 (67%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>(2 \times 10^7) (Cohort 3)</td>
<td>Bicarbonate N=10</td>
<td>1 2 7 (70%)</td>
<td>57 hrs</td>
<td>5 (50%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>(2 \times 10^7) (TOTAL)</td>
<td>N=35</td>
<td>6 2 27 (77%)</td>
<td>54 hrs</td>
<td>21 (60%)</td>
<td>8 (23%)</td>
</tr>
</tbody>
</table>

1 Diarrhea defined as:
- 1 or more loose stools (≥ Grade 3) of >300 grams
- 2 or more loose stools (≥ Grade 3) of >200 grams in a 48 hour period

2 Classification based on peak stool number or weight in a 24 hour period
- Moderate: 4-5 stools/24 hrs or 401-800 grams/24 hrs
- Severe: ≥ 6 stools/24 hrs or >800 grams/24 hrs
## Challenge Strain Shedding

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Shedding</th>
<th>GeoMean Max. Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort 1</strong>*</td>
<td>19</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Cohort 2</strong></td>
<td>15</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Cohort 3</strong></td>
<td>10</td>
<td>90%</td>
</tr>
<tr>
<td>(first challenge)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cohort 3</strong></td>
<td>10</td>
<td>90%</td>
</tr>
<tr>
<td>(second challenge)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* No difference in excretion pattern between subgroups of cohort 1
Seroconversion Rates to H10407 virulence antigens following challenge

Nearly all respond to LPS, fewer to CFA and LTB
Increase in Serum GMT anti-LPS Titers on Day 10 Following Challenge with H10407 ($\log_{10}$)
Responses to CFA and LTB

- Late-Breaker Poster for Details
- Serum responses to CFA and LTB were infrequent and low in magnitude
- ALS responses were common and higher, reflecting intestinal immune responses.
- Peak ALS responses were generally on day 7
Summary

- Combined data validate that ETEC H10407 10^7 cfu with overnight fast induces:
  - Longer incubation period
  - Reproducible AR ≥75%
  - Similar disease severity as higher dose models

- Change in fasting conditions does not alter induction of protective immunity

- Homologous protection confirmed with lower dose model

- Re-challenge data provide opportunity to further explore antigenic determinants of immunity

- Very high and consistent serological responses to LPS, less vigorous responses to CFA and LTB
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