Progress in the Development of
a Vi-CRM Conjugate Vaccine

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Vi Polysaccharide

- Vi Polysaccharide derived from *Citrobacter freundii* sensu lato
- BSL-I, rapid growth, high yields
- Vi PS NMR identical to *Salmonella Typhi*
Purified Vi Polysaccharide

Purified Vi-PS meets WHO TRS requirements
- NMR
- O-Acetyl Content
- Size
- % Pr
- %NA
- Endotoxin
- Residual reagents

SEC-HPLC

$M_{av} \approx 200-300\text{kD}$
rCRM$_{197}$

- Developed at BioE using *E. coli* host
- Process demonstrated to be robust, meets yield criteria
- rCRM$_{197}$ meets all quality criteria
Vi-CRM$_{197}$ Conjugation Kinetics

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Process step</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CRM$_{197}$ Derivatization</td>
</tr>
<tr>
<td>2</td>
<td>CRM$_{197}$-ADH Purification</td>
</tr>
<tr>
<td>3</td>
<td>Vi activation</td>
</tr>
<tr>
<td>4</td>
<td>Conjugation</td>
</tr>
<tr>
<td>5</td>
<td>Depth filtration of Conjugation Mixture</td>
</tr>
<tr>
<td>6</td>
<td>Vi-CRM$_{197}$ Conjugate Purification</td>
</tr>
<tr>
<td>7</td>
<td>Buffer Exchange of Vi-CRM$_{197}$ Conjugate</td>
</tr>
<tr>
<td>8</td>
<td>0.22 µm Filtration</td>
</tr>
</tbody>
</table>
## Vi-CRM Conjugates: Critical to Quality

<table>
<thead>
<tr>
<th>Bulk Conjugate</th>
<th>Formulated Bulk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Identity</td>
<td>• Identity</td>
</tr>
<tr>
<td>• Vi Concentration</td>
<td>• Vi Concentration (25 μg/0.5 mL)</td>
</tr>
<tr>
<td>• Vi:CRM ratio</td>
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</tr>
<tr>
<td>• Size</td>
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</tr>
<tr>
<td>• % free PS</td>
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</tr>
<tr>
<td>• O-Acetylation level</td>
<td>• Sterility</td>
</tr>
<tr>
<td>• Residual reagents</td>
<td>• Osmolarity, pH</td>
</tr>
<tr>
<td>• Endotoxin</td>
<td>• Stability</td>
</tr>
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<td></td>
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</table>
ID of Vi and CRM\textsubscript{197} in Conjugate by Dot Blot

**Identification of CRM\textsubscript{197} in conjugate**

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>ViPS</th>
<th>CRM\textsubscript{197}</th>
<th>Blank</th>
<th>Vi_CRM\textsubscript{197} (Conj)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expected</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Obtained</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
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**Identification of Vi in conjugate**

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Balb/c Mice Immunization Plan for TCV

**Study Plan:**
1. **Mice:**
   - Inbred Balb/C Female SPF Mice
   - < 6 weeks old
   - 20 mice/per group
2. **Route:** Subcutaneous
3. **Dose:** 2.5µg vaccine/100µl, 3 doses
4. **Sera collected by terminal bleeding**

**Samples Evaluated:**
1. BE Vi-rCRM
2. BE Vi-rCRM
3. Vi PS negative control (BE Vi)
4. Vi-conjugate positive control
5. Vi PS negative control (native)
6. PBS Placebo

**Responses Evaluated:**
- Anti-Vi IgG (Fold increase over Placebo and over PS only)
- Booster Effect

**Day:** 0 1 14 21 28

**Immunization:**
1. 1
2. 2
3. 3

**Sera:**
- Pre
- Post-I
- Post-III

**Dot-Blot, ELISA and SBA**
B.3 Nonclinical immunogenicity studies

Immunogenicity studies in animal models should be conducted because they provide valuable proof-of-concept information that can be used to support a clinical development plan. In addition, immunogenicity data derived from appropriate animal models are useful in establishing the immunological characteristics of the Vi polysaccharide conjugate product, and may guide the selection of doses, schedules and routes of administration that will be evaluated in clinical trials. To ensure immunogenicity in nonclinical testing weaning mice (younger than 6 weeks) should receive intramuscularly 2 injections 2 weeks apart of the conjugate vaccine and Vi should be used for a control group. Anti-Vi IgG should then be measured. The conjugate should induce a response that is at least four times higher than the response induced by Vi, and a booster response should occur after the second dose (100). Immunogenicity studies of Vi polysaccharide conjugates have been conducted in mice (71, 93, 113–115); in humans, correlation has been made between the level of anti-Vi IgG and protection against clinical disease (53, 116). Therefore, the primary endpoint for nonclinical studies of the immunogenicity of Vi conjugate vaccines should be the level of anti-Vi elicited.
Mouse Data (Post 3rd Dose)
Fold-increase over Placebo

Fold IgG Increase over Placebo

Pooled sera

Fold IgG Increase over Placebo

Individual

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Fold IgG Increase over Placebo

Pooled sera

Fold IgG Increase over Placebo

Individual
Anti-Vi IgG ELISA: Fold Increase Over PS Baseline

Samples
1. BE Vi-rCRM Lot 1
2. BE Vi-rCRM Lot 2
3. BE Vi PS control
4. Vi-CJ positive control
5. Vi PS negative control

Conclusions:
1. Significant (30X) increase in anti-Vi IgG levels observed for both Vi-rCRM samples when compared to PS baseline.
2. BE product meets the 4X threshold mentioned in WHO TRS.
Evidence of Booster Response – Dose I and III

Fold-increase in IgG

- Post-Dose I
- Post-Dose III

Fold-increase over placebo baseline
Initial Immunogenicity Evaluation
Vi-rCRM$_{197}$: Conclusions

• BE Vi-rCRM is highly immunogenic in mice. BE Vi-rCRM preclinical immunogenicity results meet WHO TRS requirements.

• BE Vi-rCRM elicits a booster response in mice.

• BE Vi-CRM conjugate have similar immunogenicity to other reported conjugates
  ➢ Vi-CRM by NVGH
  ➢ Vi-rEPA by Szu et al
  ➢ Vi-rCRM by Eubiologics
  ➢ Vi-DT by IVI
Next Steps

• BioE Vi-CRM targeted to be in clinical trials in 1Q16

• Additional lots under preparation for preclinical immunogenicity evaluation in mice and rabbit models

• BioE also working on bivalent TCV candidate vaccine (Vi-CRM and O:2-CRM). Preliminary preclinical immunogenicity evaluation ongoing.