Pre-clinical Immunogenicity of Typhoid (Vi-CRM$_{197}$), Paratyphoid A (O:2- CRM$_{197}$) and Bivalent (Vi-CRM$_{197}$+O:2-CRM$_{197}$) Conjugate vaccine

Presented by

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Summary of Presentation:

• Background: Disease epidemiology and history
• Review on pre-clinical immunogenicity models used for Typhoid & Paratyphoid A vaccines
• Biological E approach to develop Bivalent (Typhoid and Paratyphoid A) conjugate vaccine
• Preclinical Immunogenicity of Bivalent TCV:
  • Immunogenicity of Bivalent Vaccine in Mice
  • Immunogenicity of Bivalent Vaccine in Rabbit
• Current status of the Bivalent conjugate Vaccine project
• Conclusion and future perspective
Salmonella Paratyphi A : Enteric Fever
Disease burden, rationale Paratyphi A vaccine

- **Enteric Fever**
  - > 27,000,000 cases in 2000
  - *S. Paratyphi A* & *S. Typhi* cause similar disease
  - 1:1 ratio of *S. Typhi* and *S. Paratyphi A* in some countries
  - Peak age of disease 1-3 years

- **Second leading cause of enteric fever after *S. Typhi***

- **Emerging Antimicrobial Resistance:**
  - Resistance to First-line drugs: fluoroquinolones, and third generation cephalosporins, posing therapeutic challenges.

- **No vaccines for**
  - *S. Paratyphi A* (Paratyphoid Fever)
  - No correlate of protection

- **A bivalent conjugate vaccine for Typhoid-Paratyphoid would be ideal!**

Distribution of *S. Paratyphi A*

**Ju Teh et al Int.J. Med.Sci., 2014**

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Antigenic Structure of Salmonella

Vi and O antigen were shown to be important virulence determinant of Salmonella and therefore exploited as Immunogen for vaccine development.

- **Vi Antigen**: Surface polysaccharide
- **O Antigen**: Somatic antigen, Part of LPS
- **H-Antigen**: Flagellar antigen
Various type of Typhoid conjugate vaccines were tested in preclinical models and found to be immunogenic

Confirms the potential of Vi antigen as vaccine for S. typhi
Various type of O:2-CRM conjugates were tested in Mice and found to be immunogenic

- Confirms the potential of O antigen as vaccine for S. paratyphi A
Biological E Approach to develop a Bivalent vaccine to combat Typhoid & Paratyphoid

Vi
Citrobacter freundii

O:2
Salmonella Paratyphi A

Conjugation

CRM_{197}

Vi-CRM_{197} + O:2-CRM_{197}

Bivalent TCV

Immunogenicity: Mice and Rabbit

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New Zealand Rabbit

Balb/C, Mice

Day:  
1  
28  
42  
56  

Immunization:  
1st  
2nd  
3rd  

Sera:  
Pre  
Post-I  
Post-II  
Post-III  

ELISA

Route of immunization: Subcutaneous (SC)

Dose of Vaccine:
- Mice: 1/10 SHD
- Rabbit: SHD

Vaccines used for immunization

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vaccine</th>
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<tbody>
<tr>
<td>1</td>
<td>Bivalent (Vi-CRM$<em>{197}$+O:2-CRM$</em>{197}$)</td>
</tr>
<tr>
<td>2</td>
<td>Vi-CRM$_{197}$</td>
</tr>
<tr>
<td>3</td>
<td>O:2-CRM$_{197}$</td>
</tr>
<tr>
<td>4</td>
<td>Vi (unconjugated)</td>
</tr>
<tr>
<td>5</td>
<td>O:2 (Unconjugated)</td>
</tr>
<tr>
<td>6</td>
<td>PBS</td>
</tr>
</tbody>
</table>

Anti-Vi IgG was measured in the groups with Vi and Anti-O:2 IgG response was evaluated in O:2 containing groups.
A significant increase in the Anti-Vi antibody observed in monovalent conjugate (Vi-CRM$_{197}$) and Bivalent group when compared with group immunized with only Vi.

Following the second injection, a significant secondary antibody response has been observed in Both Monovalent and Bivalent vaccines.
Immunogenicity of Bivalent (Vi-CRM$_{197}$ + O:2-CRM$_{197}$) vaccine in Balb/C Mice: Anti-O:2 IgG response

- A significant increase in the Anti-O:2 antibody observed in monovalent conjugate (O:2-CRM$_{197}$) and Bivalent group when compared with group immunized with only O:2.

- Following the second injection, a significant secondary Antibody response has been observed in Both Monovalent and Bivalent vaccines.

<table>
<thead>
<tr>
<th>P1: Post Dose 1 (D28)</th>
<th>P2: Post Dose 2 (D42)</th>
<th>P3: Post Dose 3 (D56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O:2</td>
<td>O:2-CRM$_{197}$</td>
<td>O:2-CRM$_{197}$</td>
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<td>O:2-CRM$_{197}$</td>
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</tr>
<tr>
<td>Bivalent</td>
<td>Bivalent</td>
<td>Bivalent</td>
</tr>
</tbody>
</table>

Fold change in Anti-O:2 IgG over P5
Immunogenicity of Bivalent (Vi-CRM$_{197}$ + O:2-CRM$_{197}$) vaccine in Rabbits: Anti-Vi IgG response

- A significant increase in the Anti-Vi antibody observed in monovalent conjugate (Vi-CRM$_{197}$) and Bivalent group when compared with group immunized with only Vi.

- Following the second injection, a significant secondary Antibody response has been observed in Both Monovalent and Bivalent vaccines.
Immunogenicity of Bivalent (Vi-CRM$_{197}$ + O:2-CRM$_{197}$) vaccine in Rabbits: Anti-O:2 IgG response

- A significant increase in the Anti-O:2 antibody observed in monovalent conjugate (O:2-CRM$_{197}$) and Bivalent group when compared with group immunized with only O:2.

- Following the second injection, a significant secondary Antibody response has been observed in Both Monovalent and Bivalent vaccines.
Conclusion and Future Perspectives

• A Novel Bivalent conjugate vaccine containing Vi and O:2 polysaccharide was developed and found to be immunogenic in Mice and Rabbits

• No interference between Anti-Vi and Anti-O:2 antibody was observed when Vi-CRM and O:2-CRM conjugate vaccine administered together in Bivalent vaccine

• The Pre-Clinical Toxicological (PCT) study is planned and to be executed to evaluate the safety parameters of Bivalent vaccine
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