Development of a conjugate vaccines for enteric fever

8th International Conference on Typhoid Fever and Other Invasive Salmonelloses

Laura B. Martin, Head of Development Program

Dhaka, Bangladesh  1 March 2013
NVGH enteric fever vaccine strategy

*Bivalent vaccine against S. Typhi and S. Paratyphi A*

- Glycoconjugate combination vaccine
  - Building on NVGH development of Vi-CRM$_{197}$
  - Employing Novartis know-how and expertise

- S. Paratyphi A component
  - Serovar specific O-antigen, O:2
  - Covalently linked to CRM$_{197}$

- Bivalent vaccine
  - VI-CRM$_{197}$ + O:2-CRM$_{197}$

**O:2 of S. Paratyphi A**

External portion of Lipopolysaccharide (LPS)
Bivalent vaccine development since Kilifi Kenya

Building on Vi-CRM\textsubscript{197} and other NVGH projects

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>1st out-licensing</td>
</tr>
<tr>
<td>2009</td>
<td>Inauguration</td>
</tr>
<tr>
<td>2010</td>
<td>March</td>
</tr>
<tr>
<td>2011</td>
<td>Jan 7\textsuperscript{th} international conference on typhoid fever and other invasive Salmonelloses</td>
</tr>
<tr>
<td>2012</td>
<td>Tomorrow</td>
</tr>
</tbody>
</table>

**O:2-CRM\textsubscript{197} and bivalent highlights**

- € Wellcome Trust
- NVGH proof-of-concept development and non-clinical studies
- In-house optimization and scale-up in preparation for technology transfer
NVGH enteric fever vaccine, bivalent conjugate

**Unique attributes**

- Uses readily available, scalable GMP materials and equipment
- $O:2$-$\text{CRM}_{197}$ designed to be compatible with $\text{Vi-CRM}_{197}$

<table>
<thead>
<tr>
<th>Process area</th>
<th>$O:2$-$\text{CRM}_{197}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$O:2$ source</strong></td>
<td>Drug sensitive for safety</td>
</tr>
<tr>
<td>MOGM S. Paratyphi A</td>
<td>Genetically modified to produce more membrane</td>
</tr>
<tr>
<td></td>
<td>Grows well in defined, simple media</td>
</tr>
<tr>
<td><strong>$O:2$ purification</strong></td>
<td>Efficient <em>in situ</em> extraction method</td>
</tr>
<tr>
<td>No column chromatography</td>
<td>No hazardous or expensive reagents or intermediates</td>
</tr>
<tr>
<td><strong>Carrier protein</strong></td>
<td>Mutant diphtheria toxin</td>
</tr>
<tr>
<td>CRM$_{197}$</td>
<td>(used by Novartis Vaccines and Diagnostics)</td>
</tr>
<tr>
<td><strong>Conjugation</strong></td>
<td>Novel method developed by NVGH</td>
</tr>
<tr>
<td>$O:2$ per CRM$_{197}$ &lt; 2</td>
<td></td>
</tr>
<tr>
<td><strong>Cost of Goods</strong></td>
<td>Similar to Vi-CRM$_{197}$</td>
</tr>
<tr>
<td><strong>Bivalent formulation</strong></td>
<td>No overt interactions observed</td>
</tr>
</tbody>
</table>

### O:2 polysaccharide production source

*Wild-type/attenuated vs GMMA producing line*

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Wild-type</th>
<th>Attenuated</th>
<th>GMMA producing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic modifications</td>
<td>none</td>
<td>∆guaBA</td>
<td>∆TolR</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O:2 hydrolysis</td>
<td>biomass</td>
<td>biomass</td>
<td>biomass + GMMA</td>
</tr>
<tr>
<td>O:2 heterogenity HMW : MMW</td>
<td>70 : 30</td>
<td>80 : 20</td>
<td>10 : 90</td>
</tr>
<tr>
<td>Average repeating units</td>
<td>~ 45</td>
<td>&lt; 55</td>
<td>~ 25</td>
</tr>
<tr>
<td>% O-acetylation</td>
<td>~ 70</td>
<td>&gt; 70</td>
<td>~ 50</td>
</tr>
</tbody>
</table>

- MMW O:2 easier to handle gives more consistent conjugates
**O:2 polysaccharide production process**

*Optimized for 30 L scale*

<table>
<thead>
<tr>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Shake flask</td>
</tr>
<tr>
<td>• High cell density fermentation (30 L)</td>
</tr>
<tr>
<td>• O:2 antigen hydrolysis in situ (100°C, &gt; 4 h)</td>
</tr>
<tr>
<td>• Neutralization in situ</td>
</tr>
<tr>
<td>• Harvest (TFF Microfiltration)</td>
</tr>
<tr>
<td>• Ultrafiltration (TFF 30 kDa cut-off)</td>
</tr>
<tr>
<td>• Precipitation 1 (pH 3)</td>
</tr>
<tr>
<td>• Centrifugation</td>
</tr>
<tr>
<td>• Negative chromatography (Sartobind S)</td>
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<tr>
<td>• Precipitation 2 (EtOH + CaCl₂)</td>
</tr>
<tr>
<td>• Centrifugation</td>
</tr>
<tr>
<td>• Ultrafiltration (TFF 10 kDa cut-off)</td>
</tr>
<tr>
<td>• Filtration (0.2 μm)</td>
</tr>
<tr>
<td>• <strong>Purified O:2 antigen Intermediate (Bulk)</strong></td>
</tr>
</tbody>
</table>

Exploiting sterilize in place vessel

Micoli et al, Anal Biochem 2013

Lipid A

Core region

Par

n

Gal

Glc

Man

Rha

KDO

Site of hydrolysis

Micoli et al, Anal Biochem 2013
O:2 polysaccharide production process

Optimized for 30 L scale

- Shake flask
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- O:2 antigen hydrolysis in situ (100°C, > 4 h)
- Neutralization in situ
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- Purified O:2 antigen Intermediate (Bulk)

→ Exploiting sterilize in place vessel

- O-acetylation > 40%
- Protein < 0.5%
- Nucleic acid < 0.5%
- Endotoxin < 0.01 UI/µg

→ Good purity & well characterized

Micoli et al, Anal Biochem 2013
O:2-CRM\textsubscript{197} conjugation process

Also used in the iNTS conjugate vaccines

**O.2-CRM$_{197}$ production process**

*Optimized for 200 mg scale*

- O:2 activation
- Dried O:2 derivatized with ADH
- Purification of O:2-ADH
- Dry O:2-ADH
- O:2-ADH derivatized with SIDEA
- Purification of O:2-ADH-SIDEA
- Dry O:2-ADH-SIDEA
- Addition of CRM$_{197}$ to activated O:2-ADH-SIDEA
- Purification of O:2-CRM$_{197}$
- Filtration (0.2 μm)
- **O:2-CRM$_{197}$ Bulk Drug Substance**

→ **Selective chemistry through KDO, terminal sugar, of core**

→ **Good purity & well characterized**

- PS : Protein 1.3
- Yield CRM$_{197}$ 80 %
- O-acetylation > 40 %
O:2-CRM$_{197}$ immunogenicity

Similar responses from wild-type, attenuated and $\Delta$TolR strains

Anti-O:2 serum IgG responses are not highly dependent on

- Source of O:2 antigen
- Ratio of the MW populations in the O:2
- Level of O-acetylation

Historical study comparisons
Strain: CD-1 mice
Group size: 8
Dose: 1 µg
Route: SC, 200 µL
O:2-CRM$_{197}$ induces functional antibodies

*Increasing antibody = increased killing*

- O:2-CRM$_{197}$ produces antibodies that can kill *S. Paratyphi A*
- Impact of combining Vi-CRM$_{197}$ with O:2-CRM$_{197}$

**Serum Bactericidal Assay**
- CFU: $10^3$ bacteria (in exponential phase)
- Sera: serial diluted *Day 56*
- BRC': 10%
- Incubation time: 2 h
- Controls: ∆BRC', ∆sera → no SBA (0% killing)
Bivalent vaccine antibodies are bactericidal

**Against both Vi+ bacteria (Citrobacter) and S. Paratyphi A**

**Study Design**
- Strain: CD-1 mice
- Route: SC, 200 µL
- Group size: 8
- Immunization: days 1, 14 & 42
- Dose: 1 µg antigen
- Bled: day 56
- Vaccines: Vi-CRM$_{197}$, O:2-CRM$_{197}$ or bivalent

**Serum Bactericidal Assay**
- CFU: $10^3$ bacteria (in exponential phase)
- Sera: serial diluted Day 56
- BRC': 10%
- Incubation time: 2 h
- Controls: $\Delta$BRC', $\Delta$sera $\rightarrow$ no SBA (100% survival)

**Citrobacter killing correlates with anti-Vi levels**

**S. Paratyphi A killing correlates with anti-O:2 levels**

- Vi-CRM$_{197}$ + O:2-CRM$_{197}$ likely to provide coverage against enteric fever

12 | VADER course 1 | LB Martin | 5 Feb 2013 | NVGH's Enteric Fever Vaccines | Business Use Only
NVGH enteric fever vaccine, what is next
Reducing the risk and meeting the mission

- Proof of principle for a bivalent, Typhoid / Paratyphoid A, vaccine
  - Technical and animal immunogenicity

- Activities for 2013 and beyond
  - Prepared to transfer the robust processes to a commercialization partner
    - Vi-CRM$_{197}$ for typhoid fever
    - Bivalent (Vi-CRM$_{197}$ + O:2-CRM$_{197}$) for enteric fever
  - Support partner for further technical and clinical development

- Aiming for developing country access to enteric fever vaccine
  - Initial registration and roll out in India
  - WHO prequalification and wider distribution throughout S. Asia
Acknowledgements

Working together with collaborators and contributors

NVGH Enteric Fever Development Project Teams
Francesca Micoli, Massimiliano Gavini
Simona Rondini, Luisa Lanzilao
Giulia Bernardi
Mae Shieh, Breda Rogulj
Allan Saul, Giorgia Scapecchi

Technical Development
Vito Di Cioccio
Antonito Baccante, Emilia Cappelletti, Martina Carducci, Anna Maria Colucci, Graziella Di Salvo, Carlo Giannelli, Giulia Iannello, Filipe Marques, Federico Pippi, Ivan Pisoni, Silvia Sanzone, Luigi Sollai

Regulatory Affairs and Clinical Development
Audino Podda
Alessandra Anemona, Jochen Auerbach, Venere Basile, Qasim Khan, Elisa Marchetti, Michela Squaglia

Novartis Vaccines and Diagnostics
P. Costantino (Vaccine Chemistry), R. Rappuoli
Technical Development; Toxicology; Regulatory Affairs; Clinical Serology; Protocol Review Committee; Data Safety Management Board; Biostatics Clinical Data Management, Pharmacovigilance

cGMP Manufacturers
GenIbet Biopharmaceuticals (Portugal)
Areta International (Italy)

Clinical Partners
Volunteers, their families & trial site staff of
Aga Khan Univ, Pakistan
King Edward Memorial Hospital, India
Research Institute for Tropical Medicine, Philippines
Center for Evaluation of Vaccines, Belgium

Collaborators
CVD - Univ Maryland Baltimore
NIBSC
NICHDD/NIH
Oxford University
Univ Capetown
Univ Siena
Univ Trieste
Wellcome Trust Sanger Institute

External Funding
Sclavo Vaccines Association with grants from
- Fondazione Monte dei Paschi
- Regione Toscana
- EU 7th Framework - ADITEC
Wellcome Trust Strategic Award
Think what is possible