Development of a conjugate vaccines for enteric fever

8th International Conference on Typhoid Fever and Other Invasive Salmonelloses

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Dhaka, Bangladesh 1 March 2013

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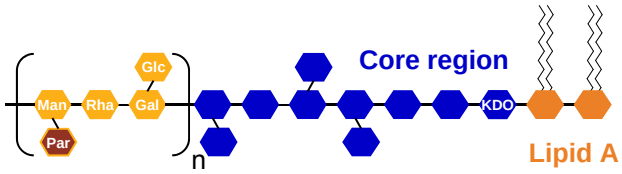
NVGH enteric fever vaccine strategy

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
  - Serovear specific O-antigen, O:2
  - Covalently linked to CRM_{197}



O:2 of *S.* Paratyphi A

External portion of Lipopolysaccharide (LPS)

- Bivalent vaccine
  - VI-CRM_{197} + O:2-CRM_{197}

Bivalent vaccine development since Kilifi Kenya

Building on Vi-CRM$_{197}$ and other NVGH projects

2008 2009 2010 2011 2012 2013

NVGH milestones

Feb Inauguration

Jan 7th international conference on typhoid fever and other invasive Salmonelloses

March

Tomorrow
1st out-licensing

O:2-CRM$_{197}$ and bivalent highlights

TG1 — **€ - Wellcome Trust** — TG2 — TG3

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

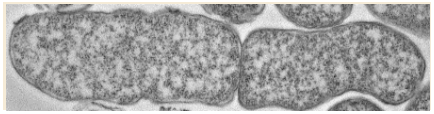
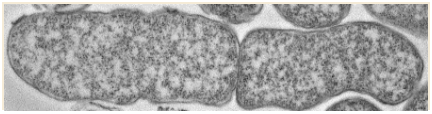
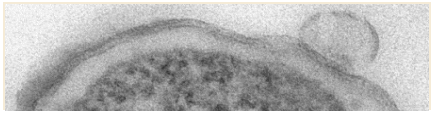
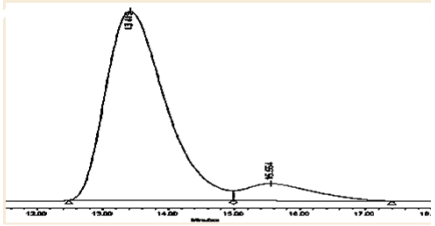
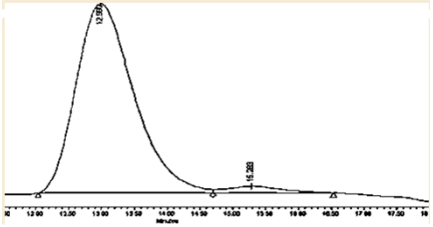
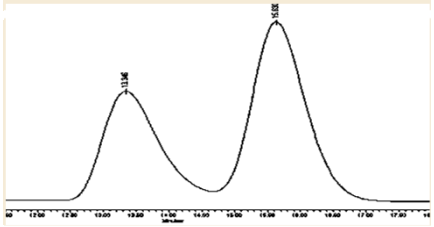
Micoli et al, PLoS One 2012 & Micoli et al, Anal Biochem 2013

- Uses readily available, scalable GMP materials and equipment
- O:2-CRM_{197} designed to be compatible with Vi-CRM_{197}

Process area	O:2-CRM_{197}
O:2 source MOGM *S.* Paratyphi A	Drug sensitive for safety Genetically modified to produce more membrane Grows well in defined, simple media
O:2 purification No column chromatography	Efficient *in situ* extraction method No hazardous or expensive reagents or intermediates
Carrier protein CRM_{197}	Mutant diphtheria toxin (used by Novartis Vaccines and Diagnostics)
Conjugation O:2 per CRM_{197} < 2	Novel method developed by NVGH
Cost of Goods	Similar to Vi-CRM_{197}
Bivalent formulation	No overt interactions observed

O:2 polysaccharide production source

Wild-type/attenuated vs GMMA producing line

Character-ization	Wild-type	Attenuated	GMMA producing
Genetic modificaitons	none	Δ*guaBA*	Δ*TolR*
Morphology			
O:2 hydrolysis	biomass	biomass	biomass + GMMA
O:2 heterogenity HMW : MMW	70 : 30	80 : 20	10 : 90
			
Average repeating units	~ 45	< 55	~ 25
% O-acetylation	~ 70	> 70	~ 50

- MMW O:2 easier to handle gives, more consistent conjugates

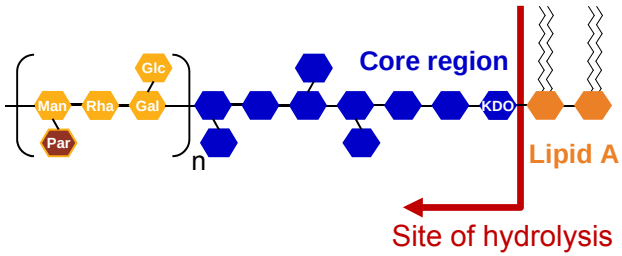
O:2 polysaccharide production process

Optimized for 30 L scale

Micoli et al, Anal Biochem 2013

- Shake flask
- High cell density fermentation (30 L)
  - O:2 antigen hydrolysis in situ (100°C, > 4 h)
  - Neutralization in situ
- Harvest (TFF Microfiltration)
- Ultrafiltration (TFF 30 kDa cut-off)
- Precipitation 1 (pH 3)
- Centrifugation
- Negative chromatography (Sartobind S)
- Precipitation 2 (EtOH + $CaCl_2$)
- Centrifugation
- Ultrafiltration (TFF 10 kDa cut-off)
- Filtration (0.2 µm)
- **Purified O:2 antigen Intermediate (Bulk)**

→ **Exploiting sterilize in place vessel**



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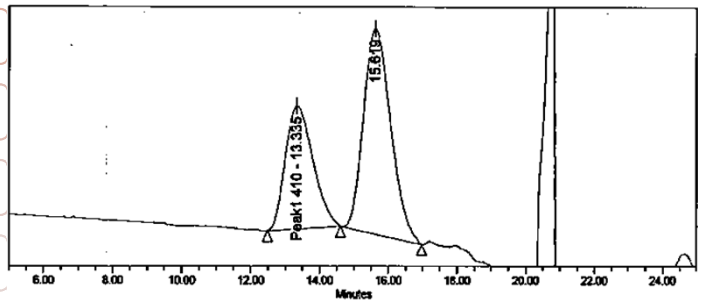

- O-acetylation > 40 %
- Protein < 0.5 %
- Nucleic acid < 0.5 %
- Endotoxin < 0.01 UI/µg

→ **Good purity & well characterized**

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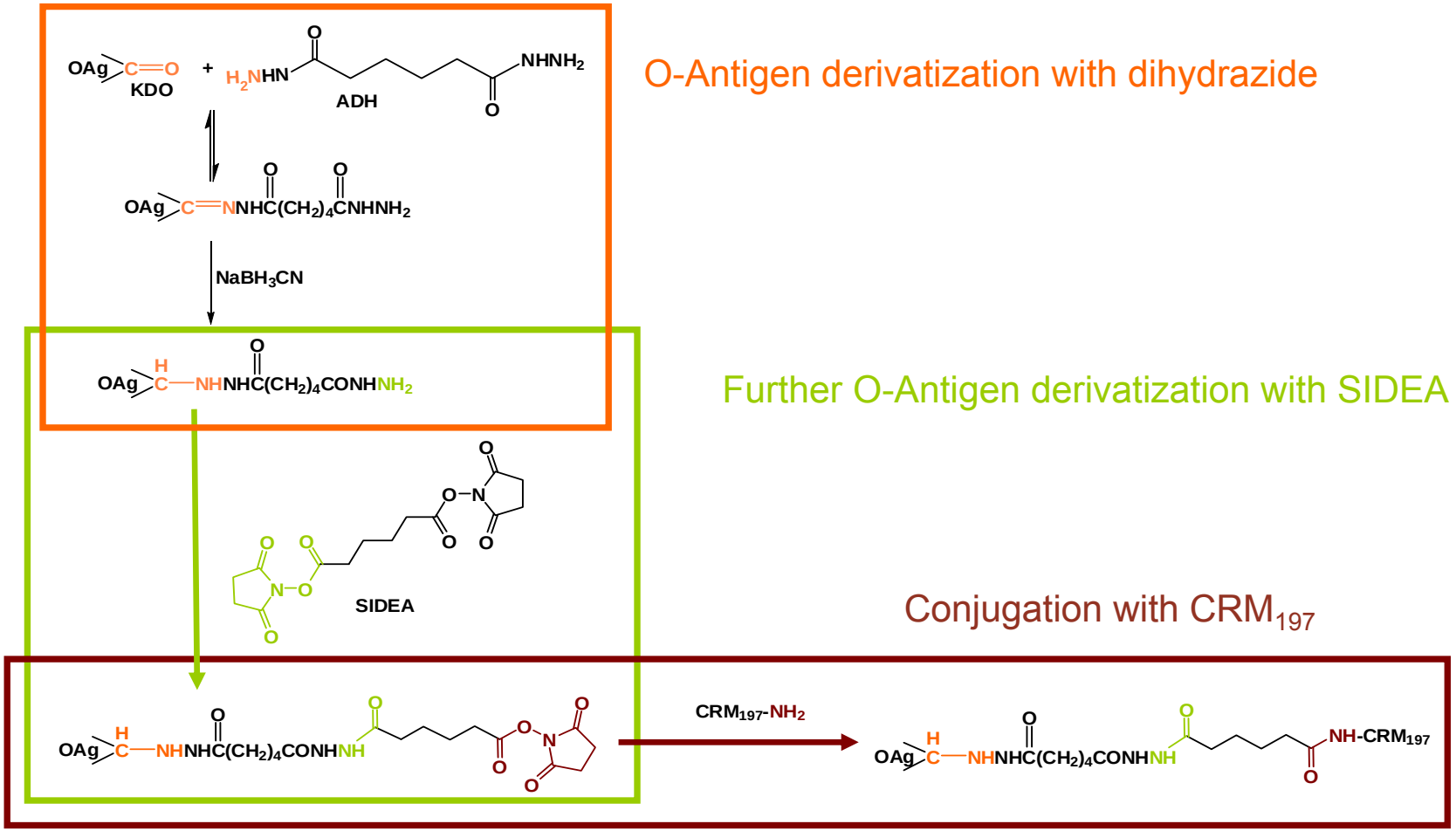
O:2-CRM$_{197}$ conjugation process

Also used in the iNTS conjugate vaccines

Micoli et al, PLoS One 2012



O-Antigen derivatization with dihydrazide

Further O-Antigen derivatization with SIDEA

Conjugation with CRM$_{197}$

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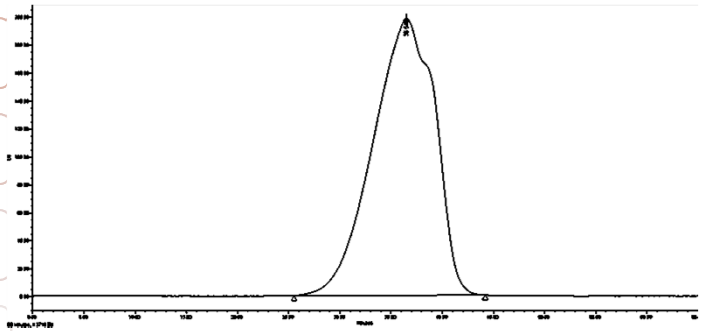
O.2-CRM_{197} production process

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**

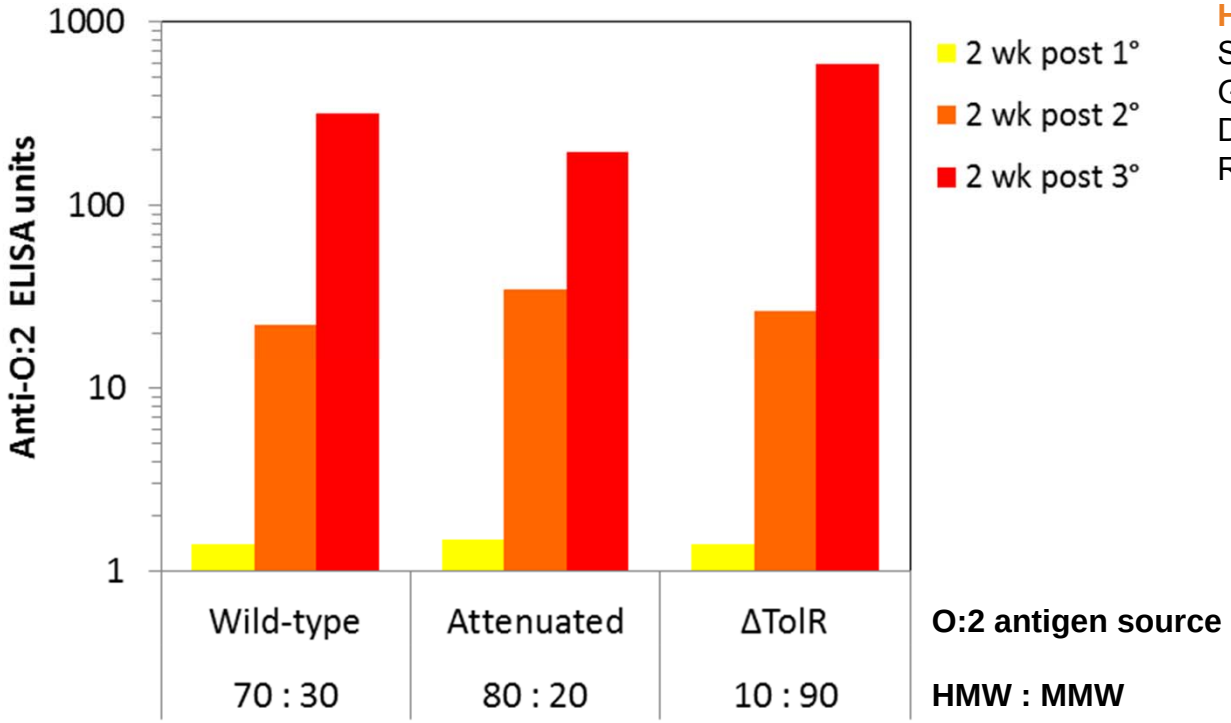


- PS : Protein 1.3
- Yield CRM_{197} 80 %
- O-acetylation > 40 %

→ **Good purity & well characterized**

O:2-CRM_{197} immunogenicity

Similar responses from wild-type, attenuated and ΔTolR strains



Historical study comparisons

Strain: CD-1 mice
Group size: 8
Dose: 1 µg
Route: SC, 200 µL

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

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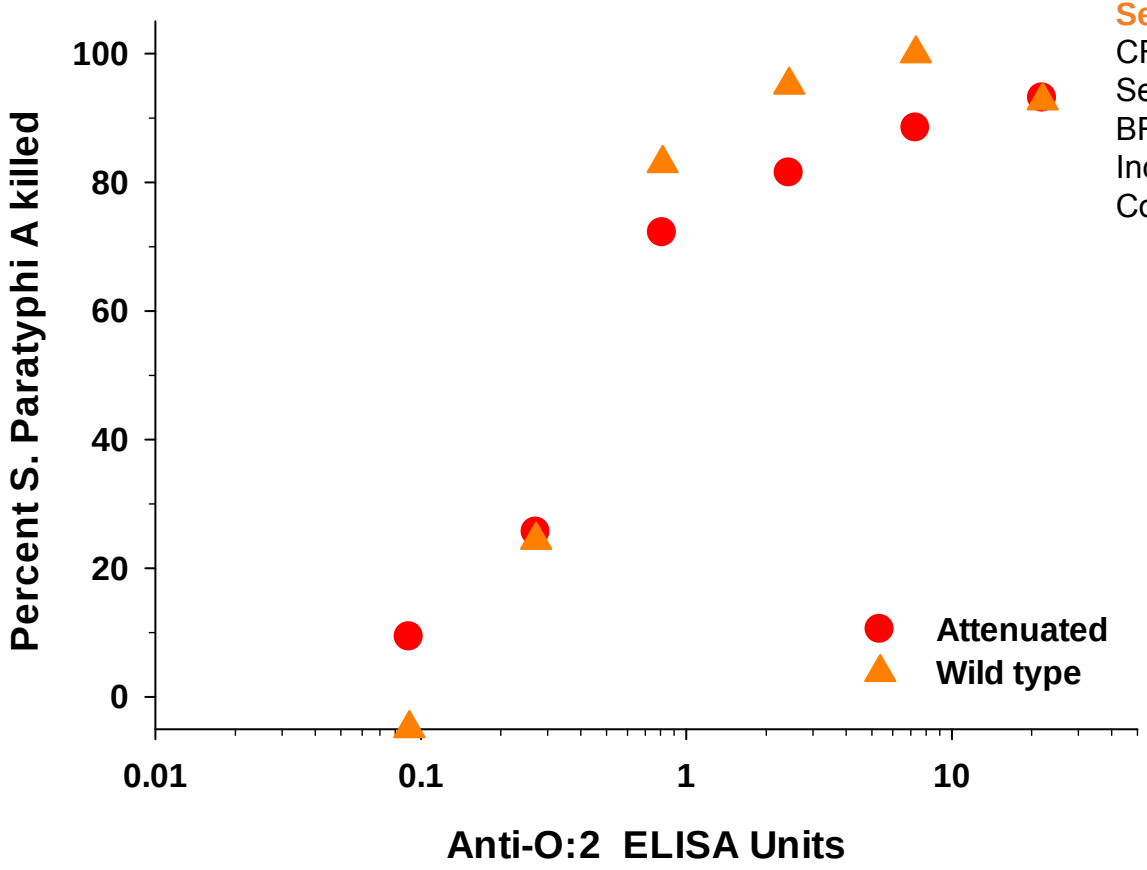
O:2-CRM_{197} induces functional antibodies

Increasing antibody = increased killing



Serum Bactericidal Assay
CFU: 10^3 bacteria (in exponential phase)
Sera: serial diluted *Day 56*
BRC': 10%
Incubation time: 2 h
Controls: ΔBRC', Δsera → no SBA (0% killing)

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

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Bivalent vaccine antibodies are bactericidal

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

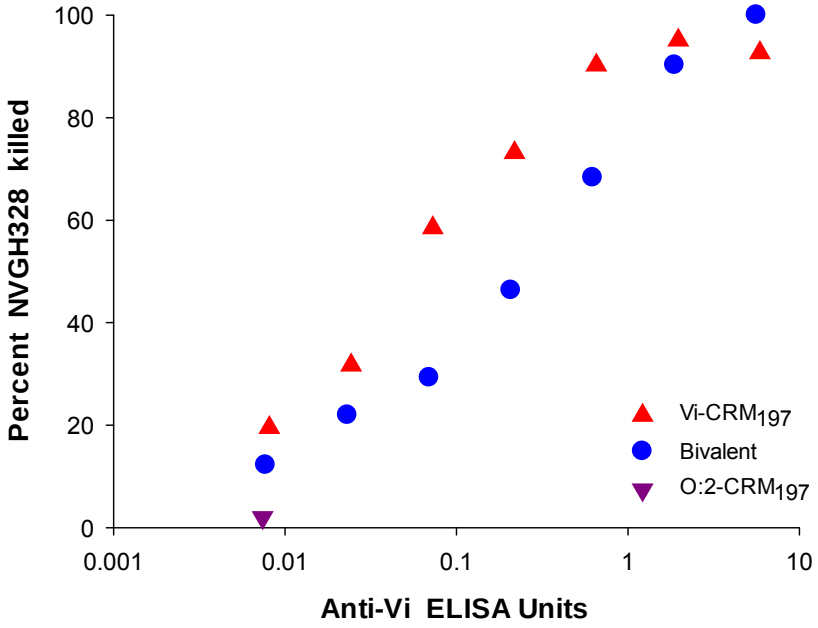
Study Design

Strain: CD-1 mice
Group size: 8
Dose: 1 µg antigen
Vaccines: Vi-CRM_{197}, O:2-CRM_{197} or bivalent
Route: SC, 200 µL
Immunization: days 1, 14 & 42
Bled: day 56
ELISA results: no immunologic interference

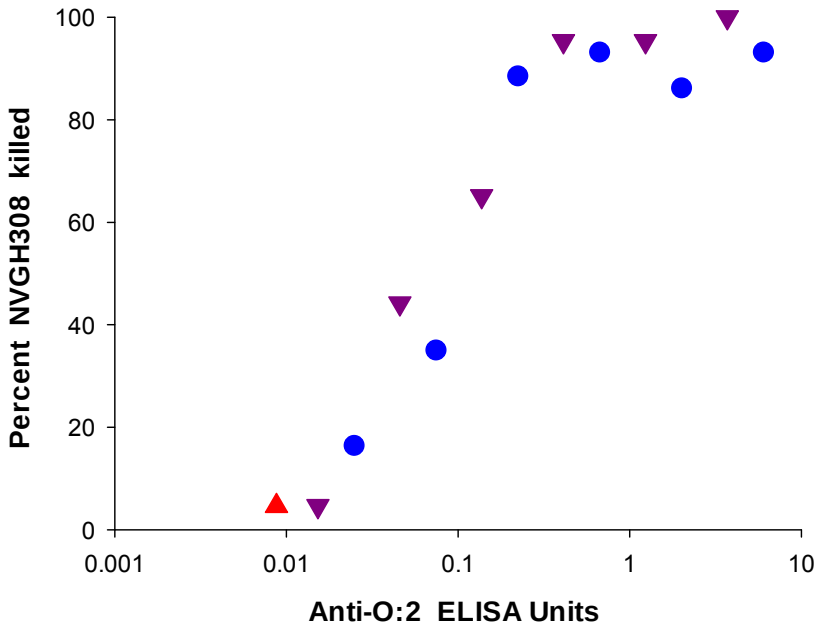
Serum Bactericidal Assay

CFU: 10^3 bacteria (in exponential phase)
Sera: serial diluted *Day 56*
BRC': 10%
Incubation time: 2 h
Controls: ΔBRC', Δsera → 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

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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
NICHD/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