Development of a vaccine based on GMMA against invasive nontyphoidal *Salmonella* disease in sub-Saharan Africa

Oliver Koeberling

10th International Conference on Typhoid and other invasive Salmonelloses
Kampala, Uganda
04 – 06 April 2017
Located in **Siena**, Italy, on the same campus as **GSK Vaccines**, one of the three GSK Vaccines R&D centers, alongside Rixensart (Belgium), and Rockville (USA)

100% owned by GSK but is a separate legal entity from GSK Vaccines

Actively seeks **partners to fund** research and development activities, particularly for **production and clinical trials**

About **50 people** in Translational Research, Technology Development, and Clinical Development & Regulatory Affairs
Invasive nontyphoidal *Salmonella*

*A major threat in Africa*

African Death Data

- iNTS: only infants
- Other diseases: all ages

Deaths per year

Sources:
- iNTS low estimate: from unpublished incidence in RTS,S malaria vaccine studies assuming 15% CFR
Invasive nontyphoidal *Salmonella*

*A major threat in Africa*

- iNTS disease occurs in whole Africa
- Almost all cases caused by serovars expressing O-antigen O:4,5 or O:9
- Human adapted, genotypes largely distinct from those responsible for gastroenteritis in industrialized countries
- African iNTS strains are highly drug resistant

- **An effective and affordable iNTS vaccine can save many lives.**

- presentation by Gianluca Bregni, Fondazione Sclavo, Siena, Italy

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**Uche V. et al., 2017**

**Marks et al., 2017**

**Kingsley R. et al., 2009**

**Bornstein et al., PLOS NTD 2017**

**Gordon M., et al., 2008**

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**Valentine Uche**, Incidence, Risk Factors and CFR of iNTS in Africa (Poster)
GVGH’s GMMA platform

Applied to different vaccines for low and middle income countries

Gram-negative bacteria naturally release small portions of the outer membrane
GVGH’s GMMA platform

Applied to different vaccines for low and middle income countries

Genetic modification to break membrane links

Gram-negative bacteria naturally release small portions of the outer membrane
GVGH’s GMMA platform

Applied to different vaccines for low and middle income countries

**GMMA**
- Just the outer bacterial layer
- Antigens presented in native environment for optimal immunogenicity
- Additional genetic modifications allow targeted vaccine design
- Simple and affordable to manufacture
- Technology applied to different pathogens (*Salmonella, Shigella, Meningococcus*)

Genetic modification to break membrane links

Gram-negative bacteria naturally release small portions of the outer membrane
O-Antigen constitutes the active component of iNTS GMMA

Induces production of functional antibodies in mice

A bivalent formulation of STmGMMA (O:4,5) and SEnGMMA (O:9) has the potential to protect against vast majority of iNTS cases in Africa
GMMA Manufacturing – Generic, Simple and Robust

*Production process established at GVGH from Shigella sonnei 1790GAHB*

**S. Typhimurium**
**S. Enteritidis**

**Genetic modifications**
1. Increase GMMA production (*tolR* KO)
2. Decrease innate system over stimulation (*msbB* and *pagP* KO)

**Fermentation**

**Purification**
- **Micro-filtration**
  - Collect 0.22 µm permeate
- **Ultra-filtration**
  - Collect 300 kD retentate

**Formulation**
- Adsorption on Alhydrogel
- **Sterile filtration 0.22 µm**

GMP lots of GMMA from both strains have been produced

**Generic Panel of GMMA Release Tests**

*Developed for Shigella GMMA and applicable for GMMA from different organisms*

### Release tests

<table>
<thead>
<tr>
<th>Test category</th>
<th>Test</th>
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<tbody>
<tr>
<td>Identity</td>
<td>O-Antigen (OAg) identification</td>
</tr>
<tr>
<td></td>
<td>Lipid A structure</td>
</tr>
<tr>
<td>Quantification</td>
<td>Total protein</td>
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<tr>
<td></td>
<td>Soluble protein</td>
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<tr>
<td></td>
<td>OAg</td>
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<tr>
<td></td>
<td>OAg molecular size distribution</td>
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<tr>
<td></td>
<td>OAg O-Acetyl</td>
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<tr>
<td></td>
<td>Lipid A</td>
</tr>
<tr>
<td>Aggregation, Integrity</td>
<td>Particle Size</td>
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<tr>
<td>Physico-chemical</td>
<td>Appearance</td>
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<tr>
<td>properties</td>
<td>pH</td>
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<td></td>
<td>Osmolality</td>
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<td>Purity</td>
<td>DNA</td>
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<tr>
<td></td>
<td>PPG</td>
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<td>Microbial status</td>
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</tbody>
</table>

### Diameter and OAg molecular size of GMMA

<table>
<thead>
<tr>
<th>Method</th>
<th>S. Typhimurium</th>
<th>S. Enteritidis</th>
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</thead>
<tbody>
<tr>
<td>Particle Size Z-Average diameter (DLS)</td>
<td>107.6 nm</td>
<td>92.2 nm</td>
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<tr>
<td>OAg Molecular size (HPLC-SEC)</td>
<td>34.6 kDa</td>
<td>30 kDa</td>
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</table>

**Size distribution by intensity**

![Size distribution graph]

10th CaT Conference | Oliver Koeberling | Kampala, Uganda  
4 - 6 April 2017
GMMA with engineered Lipid A have decreased potential for induction of pro-inflammatory response

As measured by IL-6 release from human blood cells

Salmonella Lipid A structure

Wild type LipidA Hepta-acylated

Mutant LipidA msbB, pagP KO Penta-acylated

IL-6 (proinflammatory cytokine) release

iNTS-GMMA with engineered lipidA

- Approximately 100-fold decreased potential to stimulate IL-6 release than GMMA with wild type LipidA
- Similar to detoxified Shigella sonnei GMMA shown to be well tolerated in clinical trials

Rossi O. et al CVI 2016
Rossi O. et al, JBC 2014
Formulated GMMA induce high Anti-OAg IgG responses in mice

- STmGMMA or SEnGMMA produced under the industrial process
- Vaccines were adsorbed on Aluminium hydroxide
- CD1 mice were immunized twice 4 weeks apart
- Sera obtained two weeks after the second immunization
Bivalent GMMA induce high Anti-OAg responses in mice
Comparable to individually formulated GMMA

GMMA Dose (OAg)

160 ng Typhimurium or Enteritidis

160 ng + 160 ng bivalent

Anti-IgG ELISA

Anti STm OAg antibody responses

Anti SEn OAg antibody responses

No evidence for interference between the two GMMA administered in combination
Antibodies against Bivalent GMMA show high functionality

*Serum bactericidal assay against S. Typhimurium and S. Enteritidis*

**GMMA Dose (OAg)**

- 160 ng Typhimurium or Enteritidis
- 160 ng + 160 ng bivalent

**High Throughput SBA (Necchi et al., 2017)**

- Small quantities of formulated bivalent GMMA induce antibodies with high functional activity against both, Typhimurium and Enteritidis
- Previous studies have shown that antibodies against OAg in GMMA have superior quality than antibodies against similar conjugate vaccines
Summary and Conclusion

- High burden of disease caused by iNTS in Africa
- GMMA technology
  - Applicable to different vaccines, especially suitable for low and middle income countries
  - Simple and production process
- iNTS-GMMA
  - In mice small quantities of the bivalent formulation induce high levels of antibodies with high functionality against Typhimurium and Enteritidis
  - No evidence of interference
  - Plan to proceed to GLP Toxicology study in place

- Bivalent iNTS-GMMA represent a very promising approach towards an effective and affordable vaccine against iNTS disease
- Ready for clinical proof of concept in humans
## Acknowledgments

### GVGH Project Team - current

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<tr>
<th>Name</th>
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### GVGH Early Development

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### Funding

European Union’s Seventh Framework Programme FP7/2007-2013/REA grant agreement n°251522
GMMA induce antibodies with superior functionality

Compared with OAg conjugate

% in vitro killing of Salmonella normalized for antibody concentration